THE STRUCTURE AND DEVELOPMENT OF THE CARYOPSIS IN SOME INDIAN MILLETS — IV. *ECHINOCHLOA FRUMENTACEA* LINK.

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Introduction

In the past few years embryological studies have largely been confined to the various cereals like maize, rice, wheat, oats and rye, but that of the millets such as *Echinochloa frumentacea*, *Paspalum scrobiculatum* and *Eleusine coracana* have not received adequate attention. Apart from Khosla's work (1946) on the embryology of some four Indian millets, there appears to be no other detailed account of these plants. A comparative study of the morphology and embryology of some Indian forms was, therefore, undertaken in order to record and compare points of interest, if any, with the rest of the Gramineae. The present paper is the outcome of such an effort and deals with the developmental morphology of *Echinochloa frumentacea*, the barnyard millet, belonging to the tribe Paniceae. From a perusal of the literature on the subject, it would seem that the species has not been worked out previously.

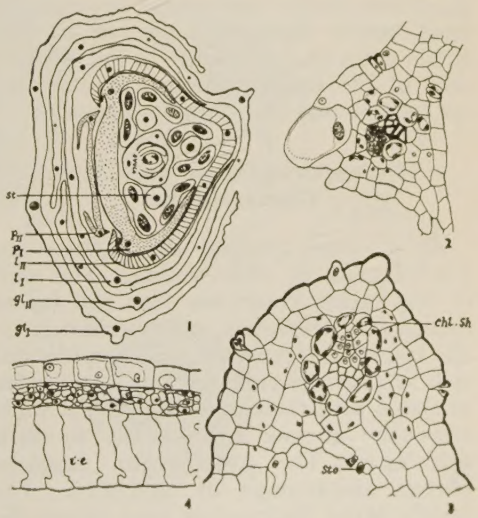
Material and Methods

The material for study was secured from plants grown at the University Botanical Garden. Spikelets before pollination, were fixed on the spot in formalin-acetic-alcohol after clipping the glumes. The ovaries were dissected out for proembryonal and later stages of growth in order to facilitate proper penetration of the fluid. Dehydration and imbedding were done in the usual way, using xylene as the clearing agent. Sections were cut 10-15 μ thick and stained in safranin-fast green.

Floral Organs

The inflorescence is a panicle of false spikes, borne alternately on a central axis. The spikelets are obovate and plano-convex in outline. Each spikelet consists of two florets of which the lower is sterile and represented only by the lemma and its palea while the upper floret is fertile and bisexual. Fig. 1 represents a cross-section of the spikelet through the fertile floret.

The lower glume (gl_I) is three-nerved and broader than long. The next glume



FIGS. 1-4 — (*chl. sh*, chlorophyllous sheath; gl_I, gl_{II}, glumes; *i.e*, inner epidermis; l_I, l_{II}, lemmas; *p*, palea; *st*, stamens; *sto*, stomata). Floral organs. Fig. 1. C.s. spikelet of fertile florets. × 90. Fig. 2. C.s. midrib region of glume I. × 205. Fig. 3. Same, of glume II. × 205. Fig. 4. C.s. palea of fertile floret. × 205.

(gl_{II}) is clasping and five-nerved with a number of secondary nerves. The lemma (l_I) is very similar in structure to the upper glume. The palea (p_{II}) is more or less reduced, hyaline and bi-keeled with ciliate margins infolded as flaps. The second lemma (l_{II}) shows typical glume structure and is five-nerved. The fertile palea (p_{II}) is bi-keeled and bears membranous wings at the back in the region of the keels. The two lodicules are cuneate in shape and fleshy. There are three stamens and two styles with plumose stigmas. The grain is broadly elliptic, flat on the dorsal and convex on the ventral side.

Figs. 2, 3 represent cross-sections of the midrib of the lower and upper empty glumes. The vascular strand is collateral endarch, and ensheathed by a chlorophyllous layer. In the region of the vascular strand it is six to seven cells thick but only three to four layered between the strands. The mesophyll cells possess but a few chloroplasts. Stomata are present on both sides of the glume with strongly cutinized guard cells. Both single-celled globular trichomes and sharp ones are abundant on the outer surface especially on either sides of the midrib. The fertile glume (l_{II}) shows similar anatomical features. A cross-section through a portion of the fertile palea (p_{II}) is shown in Fig. 4. The cells of the inner epidermal layer, (*i.e.*) are hyaline and transversely elongated.

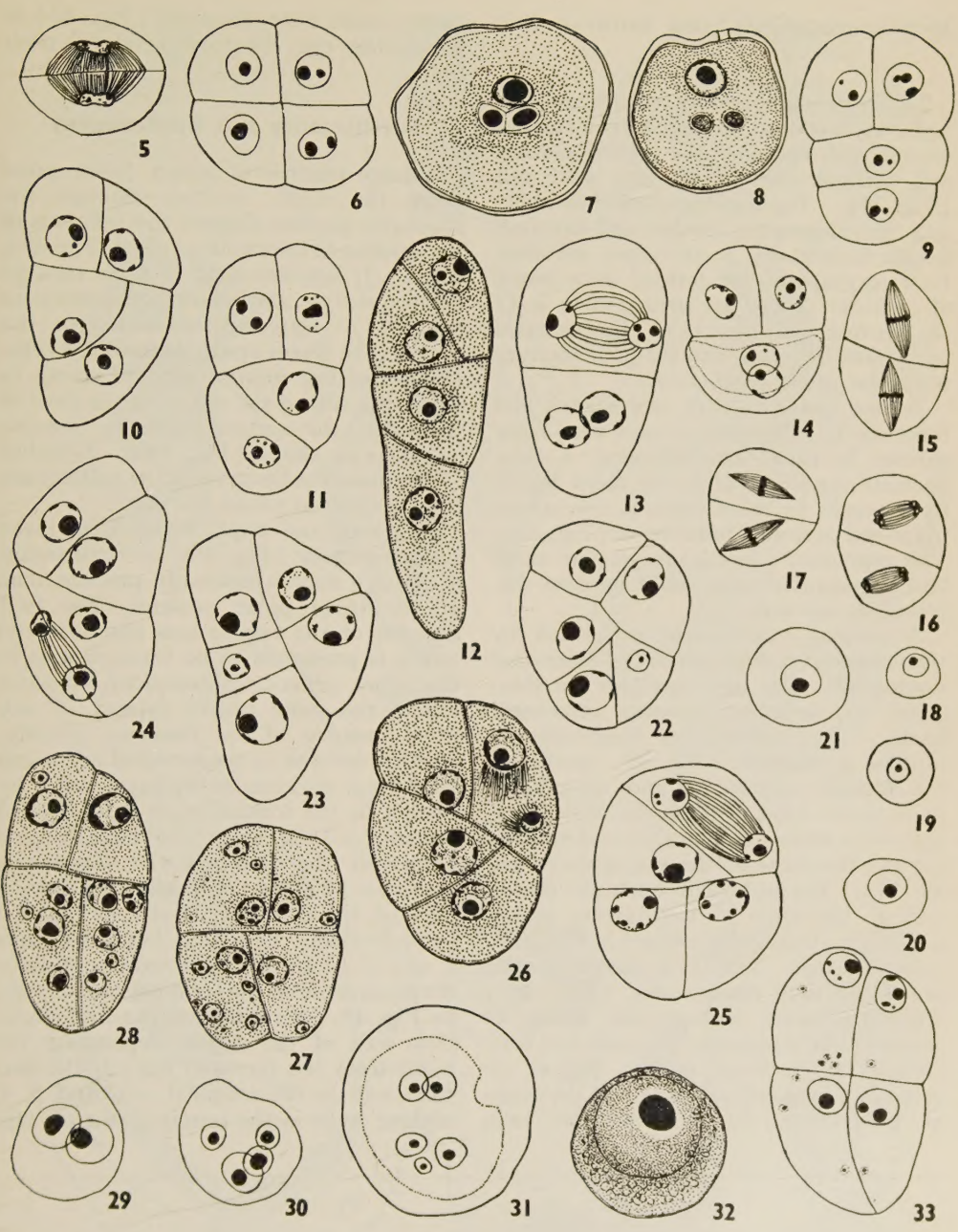
Microsporogenesis and Male Gametophyte

As in other grasses, the anthers are quadrilocular in cross-section. The wall of the anther comprises four cell layers namely, the epidermis, endothecium, middle layer and tapetum. The epidermal cells are parenchymatous and rectangular in shape with their outer tangential walls somewhat convex and remain so until dehiscence. The endothecium develops fibrous thickenings at maturity. The middle layer disorganizes even before the onset of Meiosis in the pollen mother cells. The tapetal cells are uninucleate to begin with, but later become binucleate and are of the glandular type. The two nuclei in each cell appear closely appressed to each other as if about to fuse.

Meiosis in pollen mother cells is usually normal and is of the successive type (Figs. 5, 6). The microspores separate, round themselves up and begin to enlarge. The tube and generative cells are cut off as usual. The latter is somewhat oval or more or less spindle-shaped. The tube nucleus occupies a position near the germ pore. The generative cell is freed from its wall and divides to form the two sperms (Figs. 7, 8). The mature pollen grain may contain small globular inclusions in the cytoplasm.

Occasionally, the meiotic divisions of the pollen mother cells may show no synchronization so that various stages from undivided mother cells to pollen tetrads may be found in the same locule. Other abnormalities were also observed in the course of development. Usually, the tetrads are isobilateral or tetrahedral, but T-shaped ones (Figs. 9-14) are also frequently noticed. The orientation of the spindle during the homotypic division (Fig. 15) suggests that linear tetrads may also be formed. The pollen mother cells giving rise to such tetrads are ellipsoidal or club-shaped in appearance.

In still others, oblique wall formation occurs on completion of Meiosis II in one or both the dyad cells, as indicated by the orientation of the spindle during division (Figs. 16, 17). Pollen showing size differences may be formed (Figs. 18-21). Another curious phenomenon also seems worthy of record. Secondary divisions frequently occur at the tetrad stage in one or more cells resulting in the formation of pentads (Figs. 22-25). When such divisions are not accompanied by wall formation, microspores are produced containing supernumerary nuclei (Figs. 26-31). Sometimes giant pollen grains containing fused nuclei are formed (Fig. 32). Often, however, such abnormal pollen grains degenerate. Occasionally, division in the microspore may fail to differentiate the vegetative from the generative nucleus. Thus binucleate microspores may arise. Globular inclusions (Fig. 33) are found inside the microspores at all stages of pollen development. These globules have a somewhat different staining property from those of chromatin.



FIGS. 5-33 — Microsporogenesis and male gametophyte. Fig. 5. Meiosis I. Fig. 6. Isobilateral tetrad. Fig. 7. Division of generative cell in pollen grain. Fig. 8. Three-celled pollen grain. Figs. 9-12. T-shaped tetrads. Figs. 13, 14. Tetrahedral tetrads. Figs. 15-17. Meiosis II in pollen mother cells. Figs. 18-21. Microspores of varying sizes. Figs. 22-25. Pollen pentads. Figs. 26-28. Pollen tetrads showing supernumerary nuclei. Figs. 29-31. Microspores showing supernumerary nuclei. Fig. 32. Giant pollen grain. Fig. 33. Pollen tetrad showing globules in general cytoplasm. All from acetocarmine smears. × 850.

Megasporogenesis and Embryo Sac Formation

The ovule is invested by two integuments and is anatropous. The hypodermal archesporial cell becomes the spore mother cell without cutting off any wall cell (Fig. 34). The nucellar epidermis abutting the megaspore mother cell becomes double-layered by a periclinal division. Four megaspores are formed as a result of meiosis. These are arranged in a T-shaped manner (Fig. 35). The chalazal megaspore develops into the gametophyte while the others degenerate.

In one instance three megaspores had functioned. Two embryo sacs were lying parallel to each other showing a two-nucleate condition while the third lay at right angles to these towards the micropylar end and was uninucleate (Fig. 36). The uppermost (fourth) megaspore alone had degenerated, and could be seen displaced to one side.

A normal embryo sac is formed by three successive divisions of the functional megaspore. The two synergids are elongated and pyriform showing prominent hooks. They present a longitudinally fibrillar appearance (Fig. 37) similar to the filiform apparatus, and disorganize prior to the entry of the pollen tube. The egg cell is oblong to spherical and extends beyond the level of the synergids (Figs. 38, 39). Its nucleus is centrally placed and at the time of fertilization starch grains are abundantly formed in the general cytoplasm. The three antipodal cells are large and conspicuous (Fig. 40). They are richly protoplasmic acting as reservoirs of nutriments and each cell may contain one to three nuclei. Fig, 41 represents a longitudinal section of the ovule at pollination. In one instance twin embryo sacs were observed (Fig. 42) in which the egg occupied a lateral position.

Fertilization and Embryogeny

A large number of pollen tubes grow down the style. Sections through the stigmatic papillae showed the presence of two sperms at the swollen end of the pollen tubes. It appears that in their passage down the style, the sperms are vermiform (Fig. 43). Entering the micropyle, the pollen tube forces apart the nucellar cells, penetrates the embryo sac and comes to lie by the side of the egg. At the time of discharge, the sperms appear to assume a spherical shape (Fig. 44). X-bodies arising from the burst tip of the pollen tube form a crescent round the egg.

Syngamy and triple fusion take place simultaneously (Fig. 39) or fertilization of the egg may occasionally precede that of the fused polar nucleus. The first division of the zygote was not observed but it is presumed to be transverse as in the other genera. Subsequent divisions follow the same course typical of any other member of the Paniceae, namely, vertical division in the terminal cell *a* and transverse division in the basal cell *b* resulting in the formation of a four-celled proembryo (Fig. 45). The next division occurs in the terminal tier forming a quadrant (Fig. 46). Rarely this may be delayed till after the basal cells *c* and *d* have divided. The cell *d* forms the tiers *e* and *f* (Fig. 47). Sometimes oblique divisions occur in the terminal pair of cells. In Fig. 48, *1-1* represents the first partition wall of the zygote separating the basal from the terminal tier; 2, the vertical wall in the terminal cell; and 3, 4, oblique walls in the terminal pair of cells.

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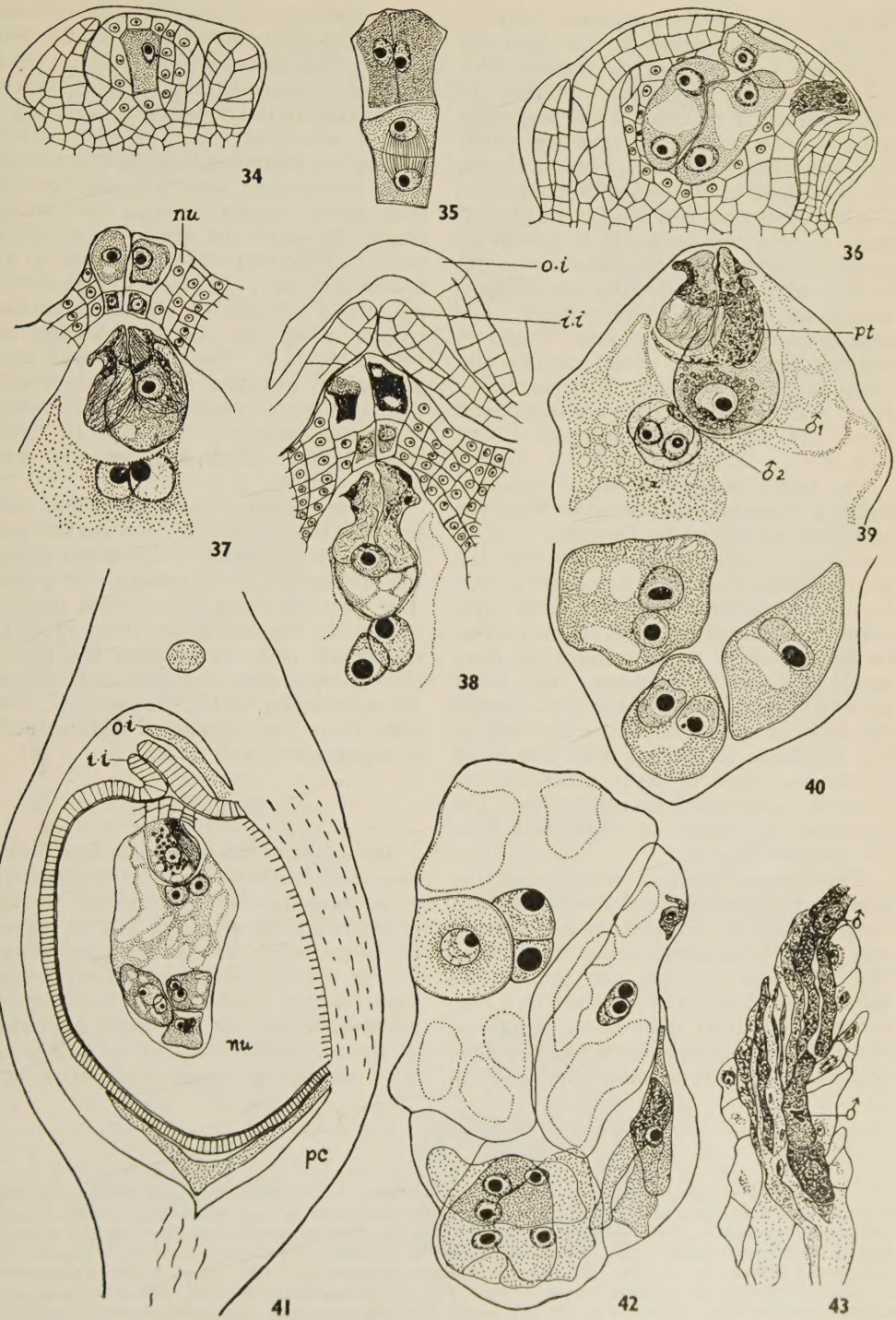
FIGS. 34-43 — (*i.i*, inner integument; *nu*, nucellus; *o.i*, outer integument; *pc*, pericarp; *pt*, pollen tube). Megasporogenesis and embryo sac. Fig. 34. Ovule showing hypodermal megaspore mother cell. Fig. 35. T-shaped megaspore tetrad. Fig. 36. L.s. ovule showing three embryo sacs and degenerated remnants of topmost megaspore. Fig. 37. Egg apparatus: synergids appear longitudinally fibrillar and nucellar cells abutting micropyle are hypertrophied. Fig. 38. Same, showing degeneration of hypertrophied cells. Fig. 39. Syngamy and triple fusion. Fig. 40. Chalazal end of embryo sac showing active antipodal cells. Fig. 41. L.s. ovary at pollination. Fig. 42. Twin embryo sacs. Fig. 43. L.s. stigmatic papillae showing pollen tubes. All × 420 except Fig. 35 which is × 810.



FIGS. 34-43.

Each of these cells undergoes another division at right angles to the previous ones so as to transform the terminal tier into an octant (Fig. 49). Division in the basal cell *d* cutting off the suspensor cell *f* may be belated till after the quadrant formation (Fig. 50). Starch grains are abundant in the proembryonal cells. A small suspensor two rows in width and three to four cells in height is formed by successive divisions of the suspensor initial *f* (Figs. 51A, B). The cells *c* and *e* contribute to the formation of the embryo. The sequence of divisions in later stages of embryo development is not regular and could not be followed with accuracy. Early periclinal divisions in the embryonal mass cut off the dermatogen (Figs. 51A, B).

The organ differentiation of the proembryo closely corresponds to that observed in the rest of the Paniceae. Fig. 52 represents a longitudinal section through the ripe grain. Initials of successive foliage leaves are formed from the stem apex within the coleoptile. The ligular process on the scutellum portion above the coleoptile is fairly conspicuous. The lower portion of the scutellum may grow beyond the level of the coleorhiza. The cells of the scutellum in contact with the endosperm are more or less rectangular to squarish constituting an epithelium. The scutellum is inserted at a distance from the plumule-sheath.

The mature embryo consists of the primary root ensheathed by the coleorhiza and the root cap, and a short axis bearing the plumule enclosed within the coleoptile. In cross-sections the protoxylem initials are arranged in the form of a ring round the central metaxylem initials of wider lumen. Longitudinal sections show that the metaxylem vessels continue upwards and turn obliquely outwards through the nodal plate terminating at the base of the stem apex (Fig. 52). Figs. 53-56 represent cross-sections of the mature embryo above the nodal plate. In Fig. 53 can be observed the horse-shoe shaped procambial strand of large size, separated from the median trace (*m. tr*) by two metaxylem vessels (*mx*). The former breaks up into the terminal scutellum trace and the two coleoptile traces at the flanks (Fig. 54). The median trace breaks up into a number of obliquely running strands supplying the first foliage leaf (Figs. 55, 56). The primary leaf has a number of principal bundles alternating with bundles of smaller size. The scutellum trace extends to the very tip and a small branch trace supplies the lower part.

Certain abnormalities were noticed which seem worthy of record. In a few instances embryo development is retarded though the endosperm development was normal. The proembryonal mass failed to differentiate its various organs (Fig. 57). The endosperm cells surrounding the globose embryo were full of starch and occupied the entire space within the pericarp. However, there was no sign of differentiation of a plumule-radicle axis.

Endosperm

The primary endosperm nucleus divides soon after formation and the nuclei occupy a parietal position embedded in thin cytoplasm surrounding a central vacuole. Divisions follow in quick succession and a large number of free nuclei are formed. Each nucleus may show two to three nucleoli. Ninety-six free nuclei are formed at the four-celled stage of the proembryo. The earlier divisions tend to occur in

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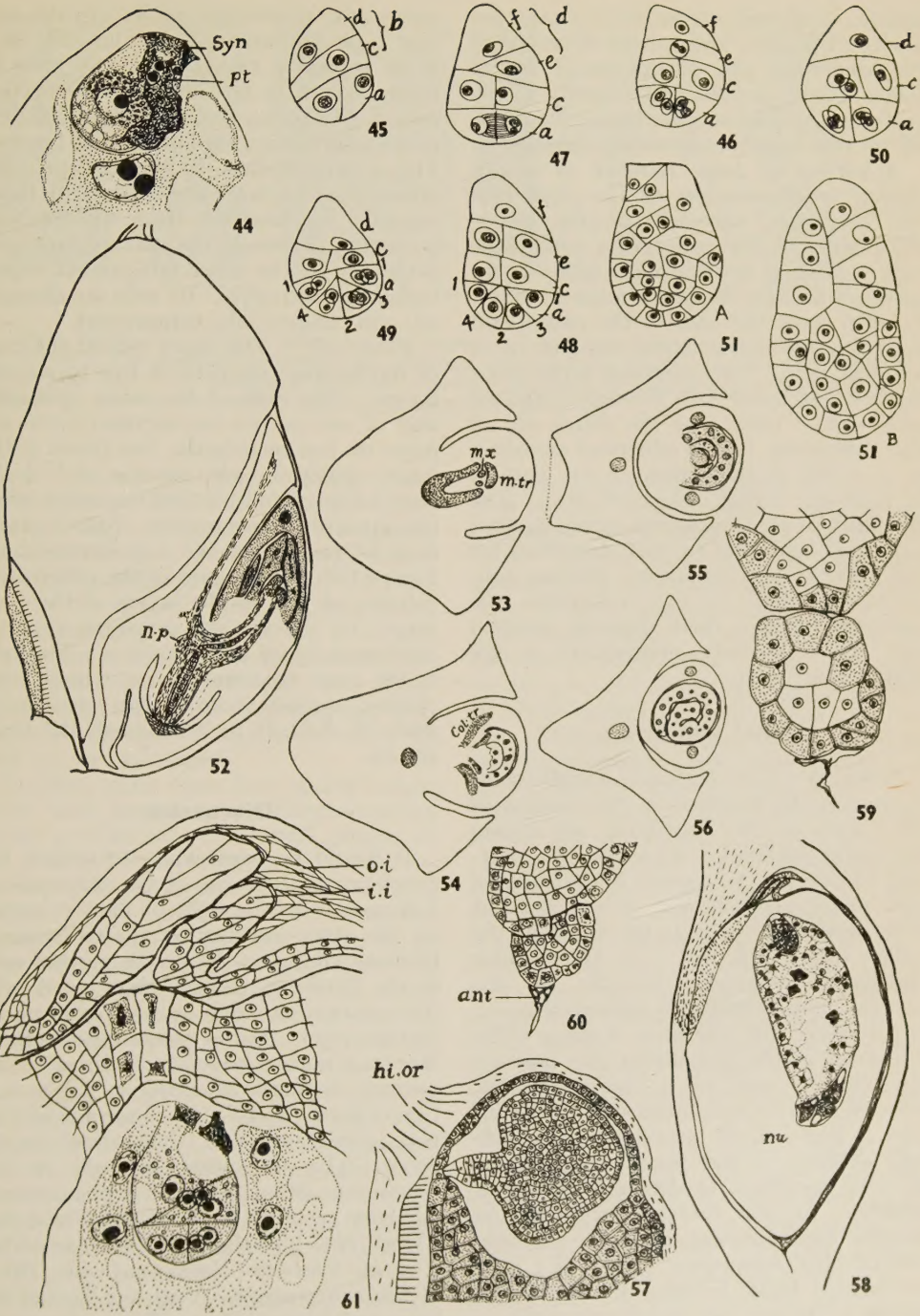
FIGS. 44-61 — (*ant*, antipodals; *col.tr*, coleoptile trace; *hi.or*, hilar orifice; *i.i*, inner integument; *m.tr*, median trace; *mx*, metaxylem; *n.p*, nodal plate; *nu*, nucellus; *o.i*, outer integument; *pt*, pollen tube; *syn*, synergid). Embryogeny. Fig. 44. Upper portion of embryo sac showing egg apparatus and undischarged pollen tube containing spherical sperms. × 400. Figs. 45-51. Stages in development of embryo. × 400. Fig. 52. L.s. mature caryopsis. × 400. Figs. 53-56. Cross-sections of mature embryo at various levels. × 40. Fig. 57. Proembryo showing non-differentiation of plumule-radicle axis. × 75. Fig. 58. L.s. ovary showing beginning of cellular phase of endosperm. Figs. 59,60. Distal end of kernel showing island of endosperm tissue. × 40. Fig. 61. Micropylar portion of ovule showing collapse of outer integument. × 400.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FIGS. 44-61.

unison. Cell walls are formed between the nuclei (Fig. 58) and further divisions of the epidermal and subepidermal layers follow, filling up the entire cavity of the embryo sac. The nucellar tissue is consumed by the rapidly expanding endosperm.

A survey of large number of ovules during transformation of the pistil into the caryopsis, reveals that the outermost layer of the endosperm may show only a limited meristematic activity or, as in some cases, it may remain inactive. In such cases the cells of the endosperm including its surface layer, tend to be of uniform size. The aleurone layer differentiates as usual with strongly cutinized cells walls. Lobing at the distal end of the endosperm is also observed probably as a result of involution of the surface layer of the kernel (Figs. 59, 60). The structure of the endosperm in the mature caryopsis conforms to that described for the rest of the Paniceae. During progressive growth of the endosperm the antipodals retain their chalazal position but are completely obliterated in the mature caryopsis.

Seed and Fruit

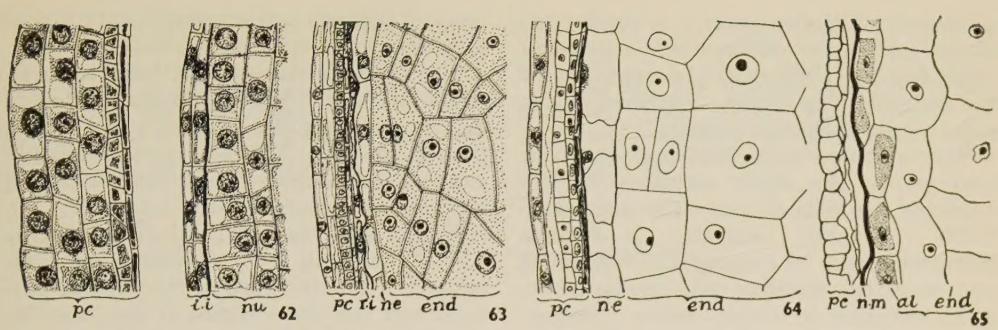
Nucellus — At the time of embryo sac formation the nucellus is two-layered in the region of the micropyle and many layered at the sides. A pair of cells abutting the micropyle appears hypertrophied and richly protoplasmic (Fig. 37) but they disorganize prior to the entry of the pollen tube (Fig. 38). As the cellular endosperm expands, collapse of the nucellar tissue except its epidermal layer, is complete. At the time of organ differentiation of the proembryo, the nucellar epidermis is seen as a layer of empty cells whose outer tangential walls are suberized. Figs. 62-65 represent longitudinal sections of the peripheral portions of the caryopsis at different stages of growth. In the mature caryopsis the nucellar layer is disorganized leaving only the nucellar membrane (Fig. 65) as a continuous layer between the inner epidermis of the pericarp and the aleurone.

Seed coat — The inner integument extends beyond the summit of the nucellus and forms the micropylar canal while the outer one is arrested in its growth and just caps the micropyle (Figs. 58, 61). It is typically two-layered but may be three-layered in the integument-insertion region. The outer one is two-three layered in the insertion region but thicker beyond. The events leading to the collapse and obliteration of both correspond to those recorded for *Setaria*. However, the only persistent portion in the mature caryopsis is the rim of the inner integument which forms the micropyle. Its cells are elongated and tangentially compressed.

Pericarp — The ovary wall at the time of fertilization consists of five to six cell layers. The cells of the outer epidermis and of one or two hypodermal layers are more or less rectangular but those of the inner epidermis are tubular and of the layer adjacent to it are smaller and more or less squarish to rectangular. Starch grains may be present in the hypodermal cells. Figs. 63-65 show stages in the progressive collapse of the middle layers of the pericarp. In the mature caryopsis the two epidermal layers alone survive. The cells of the inner epidermis are so closely compressed against those of the outer as to make it difficult to distinguish the layer clearly.

Discussion

It would be worthwhile to review the developmental details of the caryopsis of *Echinochloa* in the light of data recorded for the other members of the Gramineae. Division stages in the pollen mother cells of the Paniceae, usually show good synchronization although in the species under investigation, this is sometimes lacking. Meiosis is normal but abnormalities during microsporogenesis resulting in the formation of linear, and T-shaped tetrads besides the isobilateral and tetrahedral types, occur. This is rather unusual in the Gramineae although a similar phenomenon has been recorded in *Hordeum hexastichon* (Vogl, 1947), of the tribe Hordeae and in *Eleusine coracana* (Narayanaswami, 1952) of the Eragrosteae. Vogl has figured oblique wall formation in pollen mother cells that are asymmetrical, ellipsoidal, spherical or elongated. This feature has its parallel in certain monocotyledons like



FIGS. 62-65 — (*al*, aleurone layer; *end*, endosperm; *i.i*, inner integument; *n.e*, nucellar epidermis; *nu*, nucellus; *n.m*, nucellar membrane; *pc*, pericarp; *r.i*, remnants of inner integument). Seed and fruit. Figs. 62-65. L.s. peripheral portions of mature caryopsis showing stages in collapse of inner integument, nucellus and middle layers of pericarp. × 355.

Musa (Juliano & Alcala, 1933), *Habenaria* (Swami, 1946) and *Ottelia* (Islam, 1950). Since Vogl's report of similar abnormalities in a large number of monocots like *Sagittaria montevidensis, S. chinensis, Paris quadrifolia, Lilium henryi, Yucca filamentosa* and several species of *Allium, Tradescantia, Agapanthus, Anthericum, Pistia, Colocasia* and *Spathiphyllum*, it appears that this condition is perhaps more frequent than was considered to be the case up to this time.

Further, more than four spores (may) result from secondary divisions occurring in one or more cells of a tetrad, similar to the diminutive supernumerary nuclei reported in *Poa pratensis* (Nielsen, 1945). Nygren (1946) attributes the formation of anomalous pollen grains in some Scandinavian species of *Calamogrostis* to the influence of external factors. He is of the opinion that the same plant can, depending on the phenotypic conditions, show different meiotic-mitotic relations year by year. It is quite possible that in *Echinochloa*, the abnormalities have resulted from certain external, factors leading to unbalanced genetic constitution since they were observed in fixations from fresh culms (ratoons) that had arisen from stumps left over after the primary tillers had been grazed over by cattle.

Disturbances in microspore cytology resulting in a lack of proper differentiation of the generative and the vegetative nuclei and supernumerary divisions leading to the formation of pollen grains containing two to five nuclei are also observed in *Echinochloa*. Pollen grains with supernumerary nuclei formed by division of either the vegetative or the generative nucleus have been observed in other members of the Paniceae like *Pennisetum* (Narayanaswami, 1953) and *Panicum* (author's unpublished work), *Sorghum vulgare* (Artschwager & McGuire, 1949) and in *Anthoxanthum* (Lima-de-Faria, 1947). The globular inclusions noticed during microsporogenesis correspond to that reported in *Poa* (Nielsen, 1945).

Megasporogenesis results in a T-shaped tetrad as in *Bambusa* and *Coleanthus* (Schnarf, 1926), *Bromus* (Beck & Horton, 1932) and *Poa* (Nygren, 1950). The hypertrophy of a pair of nucellar cells abutting the micropyle is also observed in *Setaria* (Narayanaswami, in press). There is evidence that occasionally, three megaspores of a tetrad are functional. This would indicate the possibility of the development of twin or triplet seedlings from supernumerary embryo sacs within the ovule.

The antipodal cells do not divide but may be coenocytic. The stages leading to fertilization and embryogeny are typical of any other grass. At fertilization the sperm nucleus appears vermiform during passage and spherical during fusion. Percival (1921), however, reports the reverse condition in *Triticum*. A peculiar feature in the mature caryopsis of *Echinochloa* is the frequent failure of the proembryo to differentiate its organs although the

endosperm fills the grain. A similar instance of embryo failure not associated with any morphological abnormalities of the endosperm has been described in *Zea mays* (Sass & Sprague, 1950).

The aleurone layer is single and in contrast to the other members of the Paniceae, its cells do not show any significant ' cambial ' activity. A definite perisperm layer of tangentially elongated and persistent nucellar cells has however, been recorded in *Oryza sativa* (Santos, 1937) and *Euchlaena mexicana* (Cooper, 1937) although Juliano & Aldama (1937) mention the occurrence of only the thick outer tangential wall of the nucellar epidermis in the Inapostol variety of common rice. Reports, however, of the retention of the nucellar epidermis as a definite layer have to be treated with caution, as it may represent a case of immature caryopsis where the cells are in the process of disorganization and collapse.

Summary

The morphology of the spikelet has been described. Microsporogenesis is normal and of the successive type. A number of abnormalities like the formation of T-shaped and linear pollen tetrads, occurrence of pentads and supernumerary nuclei in microspores have been observed.

The ovule is anatropous, bitegmic and crassinucellate. Megasporogenesis results in T-shaped tetrads. Embryo sac formation is of the Normal type. Multiple embryo sacs arise from the functioning of more than one megaspore of a tetrad.

Embryogenesis is typical of any grass. The sequence of early divisions has been traced. The structure of the mature embryo and the distribution of the vascular supply, as observed in cross-sections, have been studied. Occasional failure of the proembryo to differentiate a plumule-radicle axis is noted.

The endosperm is of the free Nuclear type. Its development follows the conventional type. The surface layer of the endosperm does not behave as a ' cambium '.

The surviving outer layers of the caryopsis forming a protective covering comprise the suberized nucellar membrane, the aleurone, the rim of the inner integument and the two epidermal layers of the pericarp.

The author wishes to extend his appreciation to Prof. P. Maheshwari for his kind guidance during the course of this investigation.

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NEW SYMBOLS IN CYTOLOGY

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In the embryological literature of the past fifteen years, the terms *reduced* and *unreduced* (Stebbins, 1941; Gustaffson, 1946-1947) have frequently been used to indicate respectively chromosome complements numerically reduced by regular meiosis, and unreduced because the meiotic process is irregular (for instance, formation of a restitution nucleus), or is absent. Thus with reference to reproduction of apomictic plants such expressions as unreduced pseudogamy, reduced parthenogenesis, etc., have been introduced. This terminology seems precise and preferable because apomictic plants are usually polyploid and in these cases it is incorrect to use the terms haploid and diploid. Furthermore, the terms reduced and unreduced are particularly suitable in cases where the degree of polyploidy is unknown.

In view of the above considerations, the author proposes the following system of symbols to indicate the various chromosome numbers, in the ontogenetic cycle of an individual without consideration of the problem of polyploidy:

I. z (zygotic number) — chromosome complement of the zygophase (sporophyte);

II. g (gametic number) — chromosome complement of the gamophase (gametophyte);

III. r (reduced number) — reduced chromosome number as observed at the end of regular meiosis (reduced number, reduced gametes, reduced parthenogenesis, etc.);

IV. $2r$ (unreduced number) — twice the number r;

V. $<r$ (hypo-reduced numbers[1]) — chromosome numbers smaller than r;

VI. $<2r$ (hypo-unreduced numbers) — chromosome numbers smaller than $2r$, more exactly between $2r$ and r;

VII. $>2r$, $3r$, $4r$, etc. (hyper-unreduced numbers) — chromosome numbers between $2r$ and $3r$, $3r$, $4r$, etc.

In addition, the author considers that the term *unreduced* should be reserved for the number $2r$ only; on the other hand, the term *non-reduced* might be used for all cases in which the reduced number does not occur.

With reference to the terms zygotic and gametic mentioned above, it may be pointed out that Jorgensen (1928) first proposed the terms gametic and somatic for the two antithetic phases of the ontogenetic cycle (the gamophase and the zygophase). Subsequently, Chiarugi (1933) put forward the more correct antithesis, gametic and zygotic. Furthermore

1. The prefixes hypo and hyper are added to the terms reduced and unreduced, in the manner usually accepted for the terms haploid, diploid, etc. Thus, in a diploid organism the hypo-haploid number strictly coincides with the hypo-reduced number and the hyper diploid with the hyper-unreduced number, etc.

it may be mentioned that most authors usually express the zygotic number as twice the gametic one. Thus Darlington (1937, p. 62) uses n for the gametic number, $2n$ for the zygotic and x, $2x$, $3x$, etc., for the polyploid series. Sharp (1943, p. 205) has adopted an opposite method: he uses x and $2x$ for the gametic and zygotic numbers respectively, and n, $2n$, $3n$, etc., for polyploids. Disregarding any preference for either of these two methods, there is no strict arithmetical connection between the gametic and the zygotic numbers. In fact, a plant with the zygotic number $2n = 15$, according to Sharp's method, will produce gametes showing the numbers $n = 8$ and $n = 7$, however, the correct numerical value of n should be $n = 7, 5$. Moreover, the gametic number may be the same as the zygotic one, for instance, where the meiotic process has been suppressed. Finally meiosis may occur regularly and still the zygotic and gametic numbers may coincide. This situation occurs in some animals such as *Dugesia* (Benazzi-Lentati, 1952), *Polycelis* (Lepori, 1950), *Eiseniella* (Omodeo, 1951). Here, before meiosis occurs a cytological process (endomitosis) doubles the somatic chromosome number. Therefore, the resulting gametes show the zygotic (somatic) number. The last example clearly shows the importance of stating whether the gametic number arises by meiosis, regular or irregular, or without meiosis. The introduction of the symbol r is precisely to satisfy such an exigency. The following cases will clarify the use of the author's terminological method. Let us suppose a plant has the zygotic number 20, i.e. $z = 20$. When the gametophyte develops by regular meiosis, we observe the reduced gametic number $g = r = 10$ and in short $r = 10$. If meiosis is accompanied by the formation of a restitution nucleus, we observe $g = 2r = 20$. Meiosis may be also of the birestitutional type. In that case the result is $g = 4r = 40$ and in short $4r = 40$. Finally for the case of somatic apospory (gametophyte development in the absence of meiosis) we will have $g = z = 20$. The above symbols and terminology are suggested when polyploidy is not involved. However, the degree of polyploidy, if known, can be easily indicated in brackets, for instance, the zygotic number of a tetraploid will be, according to Sharp's method of denoting polyploid series, $z\ (4n) = 20$. The above system of terms is simple, clear and applicable to both plants and animals.

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THE CONCEPTS OF SPORE, SPOROGENESIS AND APOSPORY

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The concept of *spore* is still indefinitely and variously defined by morphologists. Most authors consider it a " specialized cell having the capacity for directly developing into a new organism ". The literature on this subject is so extensive that it cannot be summarized in a brief article; however, the following observations are made with particular reference to recent advances in angiosperm embryology.

It appears more appropriate to restrict the term *spore* to denote " an immediate product of meiosis (regular or irregular) that directly develops into a gametophyte ", an instance of heterophasic reproduction[1]. The term *conidium* can then be reserved for all cases in which a single cell directly gives rise to a new organism similar to the parent (i.e. it reproduces the same phase of the ontogenetic cycle—homophasic reproduction). The term *gametes* will indicate cells specialized for fertilization which typically participate in fusion (amphimixis), but atypically omit this phenomenon (apomixis).

It is observed that many authors do not strictly associate the concept of spore with meiosis. However, the occurrence of meiosis is a fundamental point of distinction between sporogenesis (spory) and aposporogenesis. In fact, if the concept of spore is not associated with the occurrence of meiosis, the concept of apospory seems not to exist. Let us, for instance, call a *spore* a specialized cell of the sporophyte that directly reproduces the gametophyte. According to such definition, we would have the following terminology:

(a) Spore formation by regular meiosis, i.e. regular sporogenesis, which should be called *euspory* (abbreviation for eusporogenesis);

(b) Spore formation by irregular meiosis, i.e. irregular sporogenesis, which should be called *aneuspory* (abbreviation for aneusporogenesis);

(c) Spore formation without meiosis, including two different categories:

(i) The spore is a true spore mother cell that develops without meiosis, a phenomenon which should be called *apomeiotic spory*[2] (abbreviation for apomeiotic sporogenesis), but which is usually known to cytologists as *gonial apospory* (or *generative apospory*)[3];

1. A distinction can be drawn between heterophasic and homophasic reproduction (*see* Battaglia 1947b, 1955). Homophasic reproduction takes place, when the same phase of the ontogenetic cycle is produced, for instance a sporophyte from a sporophyte or a gametophyte from a gametophyte. Heterophasic reproduction is the production of the antithetic phase of the ontogenetic cycle, that is a sporophyte from a gametophyte, etc.

2. The term *apomeiosis* was first used nearly forty years ago by Renner (1916) to include all cases leading to the formation of a gametophyte with the unreduced chromosome number. Present cytological knowledge suggests that apomeiosis is better used in the sense of " non-occurrence of meiosis " than " non-occurrence of reduction ". In this last sense, Renner's concept seems better expressed by the term *apo-reduction*. For the above concept of apomeiotic spory, Gustafsson (1946-1947) uses the term *diplospory*. Since apomictic plants are very frequently polyploids, *unreduced spory* should be a better term than diplospory. However, the author prefers the term *apomeiotic spory* because the essence of this phenomenon is the loss of meiosis. Apomeiosis means literally non-occurrence, by loss, of meiosis and this loss is conceivable only for a cell that regularly shows meiosis, i.e. a spore mother cell. Consequently, the expression apomeiotic spory seems more appropriate.

3. *Gonial apospory*, first used by Chiarugi (1926), has priority over the widely used term *generative apospory* (Rosenberg, 1930). In

(ii) The spore is a cell other than the true spore mother cell, i.e. some cell of the sporophyte that develops without meiosis, a phenomenon which ought to be called *somatic spory* (abbreviation for somatic sporogenesis), but which is usually known to cytologists as somatic apospory or shorter apospory.

Thus it seems evident to the author that if the concept of spore is not associated with meiosis, the term *apospory* is meaningless.

On the contrary, if it is assumed that spore formation (spory) strictly involves meiosis, we must decide whether or not it is correct to speak of spores also in the case of irregular meiosis. An examination of the course of reproduction in angiosperms strongly supports the second interpretation, i.e. true spores can arise as a result both of regular and irregular meiosis. In *Rudbeckia speciosa* (Battaglia, 1946) the course of *androsporogenesis* (microsporogenesis) is regular and produces the usual pollen tetrads. Occasionally, two telophase nuclei at the end of the second division of meiosis come into contact and fuse. Thus meiosis results in one unreduced and two reduced androspores (microspores) [4]. These three pollen grains possess the same morphology and behave in a similar way. It seems clear that the product of meiosis can function as a spore whether it has the reduced or the unreduced chromosome number. In *Allium schoenoprasum* (Levan, 1936), *Datura* (Satina & Blakeslee, 1935) under natural conditions, *Tradescantia* (Sax, 1937), and *Fritillaria meleagris* (Barber, 1940) under experimentally induced conditions, the first division of meiosis in the androspore mother cells (microspore mother cells) proceeds normally, but the second is totally suppressed. The first post-meiotic division takes place when the cells are developing into uninucleate pollen grains. This division is of a peculiar type and is called *diplounivalent mitosis* (Battaglia, 1947a) and gives rise to unreduced vegetative and generative nuclei. It may be mentioned incidentally that the nuclei at the end of the first meiotic division possess chromosome types having the two-chromatid structure, i.e. halves of bivalents disjoined at Anaphase I or univalent in the wide sense. Owing to chromosome reproduction that occurs as a regular phenomenon before the first mitosis in the pollen grain, these two-chromatid structures change into four-chromatid structures, i.e. univalents become diplounivalents (Battaglia, 1947a). The unreduced pollen grains so obtained show the same morphology and behaviour as the reduced grains and are completely viable. We may assume that the process of spore formation (sporogenesis) is normally completed even if the second division of meiosis is omitted. Equally interesting and significant is the case of *Fritillaria meleagris* (Barber, 1940). In this plant at high temperatures, the first division of meiosis in pollen mother cells is arrested at diakinesis. There follows a long resting stage (suppression of almost all of Meiosis I and II) during which the androspore mother cells are developing into uninucleate pollen grains. At the prophase of the first mitosis in the androspore we can observe the exceptional chromosome structures called *diplobivalents* (Barber, 1940). These are eight-chromatid complexes. At the end of this exceptional division (a *diplobivalent mitosis*) the usual generative and vegetative cells are produced. The case of *Fritillaria* shows that sporogenesis may proceed equally well even if meiosis is nearly suppressed.

In conclusion the best definition of the spore concept seems to be the following: a spore is a cell of the sporophyte, produced by regular or irregular meiosis, that directly gives rise to the gametophyte. Accepting this interpretation

addition it is more appropriate because *generative* was previously used in the sense of haploid by Winkler (1908). Further, the concepts of somatic apospory and gonial apospory, the latter restricted to indicate the development of the spore mother cell without meiosis, were clearly defined by Fagerlind (1940) who, however, prefers the term generative.

4. The author considers *androspore* and *gynospore* to be better terms than the usual microspore and megaspore. In conformity with this usage the terms *androsporogenesis* (microsporogenesis) and *gynosporogenesis* (megasporogenesis) have been accepted (*see* in this connection Thompson, 1927; Looby & Doyle, 1942; and Doyle, 1935).

the various methods by which the sporophyte reproduces the gametophyte can be expressed by the terms *euspory*, *aneuspory*, *gonial apospory* and *somatic apospory*.

The above considerations may be supplemented by two diagrams giving evidence of the phenomena and showing their connection with gametophyte development. For this purpose some of the more outstanding examples of *euspory*, *aneuspory* and *apospory*, both in androsporogenesis and gynosporogenesis of angiosperms, have been selected. Different cases have been outlined according to the author's interpretation of the male and female gametophytes of angiosperms (Battaglia, 1951). However, the following small modifications have been introduced in the figures, for clearer presentation of the chromosome peculiarities and to facilitate comparison of the development of the male and female gametophytes:

(a) In order to make the diagrammatic representation of the sporogenesis of a wider applicability, the *dyad* step has been substituted by *interkinesis* (interk.), and *tetrad* by *spore-morphogenesis* (spore-morph.). By the latter term the author wishes to indicate the maturation of the spores. The processes involving the latter are not so prominent in the gynospores of angiosperms, but in androspores spore-

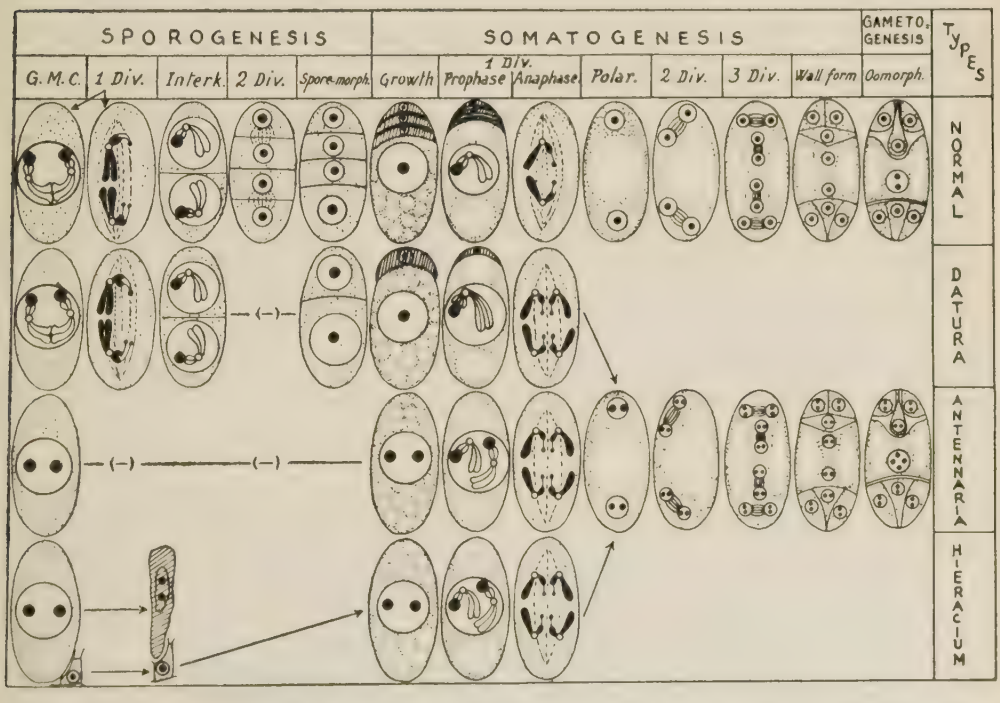

FIG. 1 — Representation of some types of sporogenesis and female gametophyte development in angiosperms. *G.M.C.*, gynospore (megaspore) mother cell; *interk.*, interkinesis; *spore-morph.*, spore-morphogenesis; *wall form.*, wall formation; *oomorph.*, oomorphogenesis. The chromosomes show chromatids, short arms, long arms, centromere (a small white circle) and a satellite on the short arm. The gynospore mother cells in the *Normal* and in *Datura* types are drawn in the diakinetic stage and show one bivalent (with terminalized chiasmata). The prophase nucleus of the first somatogenic division in the *Datura* type shows 4-chromatid structure (post-homotypic diplounivalent). For clarity of karyological details two chromosomes (and two nucleoli) have been considered to represent the unreduced (diploid) chromosome complement, thus, in nearly all drawings the number of the nucleoli indicates the chromosome complement for each nucleus. The symbol (—) stands for suppression of one division.

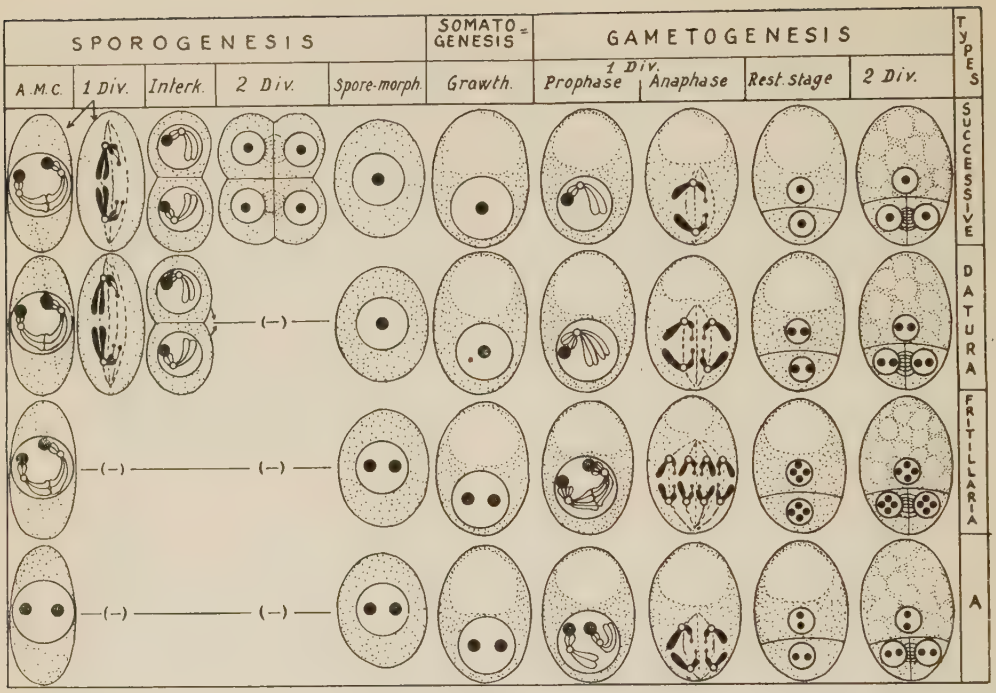

FIG. 2 — Representation of some types of sporogenesis and male gametophyte development in angiosperms. The *successive* type is based on the formation of the cell wall after each meiotic divsion. The *Datura* type was previously designated by the author as the *Allium schoenoprasum* type (Lievan, 1936), because he had not taken note of the earlier published data of Satina & Blakeslee (1935). For the *Fritillaria* type see the *Fritillaria meleagris* type in Battaglia (1951). The *A* type shows a method of development strictly parallel to the *Antennaria* type of embryo sac development, and it is so far unknown for the male gametophyte. For the explanation of chromosome structures and peculiarities, *see* the legend for Fig. 1. For detailed interpretation of the male gametophyte, *see* Battaglia (1951). Obviously the first gametogenic division represents the formation of the vegetative and generative cells and the second gametogenic division the formation of the two sperm cells. The symbol (—) stands for suppression of one division.

morphogenesis is more elaborate consisting of thickening of the membrane, sculpturing of the exine and so on. Spore-morphogenesis is an alternative process to gameto-morphogenesis for the products of meiosis. Thus in higher animals the cells arising as a result of meiosis undergo the gameto-morphogenesis phase in changing into gametes.

(b) The first step of somatogenesis, *vacuolation*, has been substituted by *growth* because the functional spore or gametophytic initial cell undergoes a true period of growth, of which vacuolation is only a manifestation.

(c) The first division of somatogenesis has been represented by prophase and anaphase to give clear evidence of the chromosome peculiarities in some types of development, as in the *Datura* and the *Fritillaria* types.

(d) The step, *resting stage*, between the second and the third division of somatogenesis has been omitted in Fig. 1 because this configuration is unnecessary when no differences in the gametophyte development are involved.

(e) Representation of male gametophyte development has been figured as independent from the phenomenon of pollen germination to avoid unnecessary complication.

(f) The sign (—) has been introduced to signify omission of a division.

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MESOZOIC PTERIDOSPERMS

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The earliest collectors of fossil plants found, in the rocks associated with coal seams, the remains of leaves which, from their shape and venation, appeared to represent ferns. When, in the 19th century, these remains were carefully studied and named it was seen that many of the commoner of the fern-like leaves were never found to bear sporangia, although other types frequently bore sporangia or synangia on their pinnules. In 1903 Oliver & Scott were able to show that certain fronds of the *Sphenopteris* type were almost certainly borne on stems found in a petrified condition and possessing several gymnospermous features, and that these plants produced seeds. This work, which was mainly due to Oliver, demonstrated the existence in Upper Carboniferous times of a group of seed-bearing plants with fern-like leaves. Subsequent work showed that other fern-like fronds bore comparable gymnospermous reproductive structures, and the discovery of allied stems and separate seeds and pollen-bearing organs indicated that this group, which had been called the pteridosperms, included many plants with a wide variety of vegetative and reproductive characters. These plants evidently formed an important constituent of the world's vegetation from

Upper Devonian times to the Lower Permian.

It was generally assumed that plants of this class became extinct at the close of the Palaeozoic period. But the older Mesozoic rocks also contained the remains of fern-like fronds which were invariably sterile. This led Seward and others to suggest that the floras of Mesozoic times also contained some pteridosperms, although no evidence of stems or reproductive structures had then been found to support this suggestion.

About forty years ago a new criterion for the separation of the true ferns from the seed plants was recognized. The leaves of the fossils referable to the Cycadales, Bennettitales, Ginkgoales and Coniferales had cuticles which resisted the action of strong acidic oxidizing agents; by suitable treatment they could be separated and mounted for examination under the microscope; they showed the shape of the epidermal cells and much of the structure of the stomata. But when fragments of fossil fern leaves were treated in the same way they dissolved completely. Some of the palaeozoic pteridosperm fronds and most of the supposed mesozoic pteridosperms prove to have cuticles like those of the other gymnosperms. The same treatment with nitric acid and potassium chlorate, followed by dilute alkaline solutions, reveals the cutinized membranes in fossil seeds and pollen-bearing structures; the form of the pollen grains can be determined since their extines were cuticularized. By comparing the form, size and appearance of the epidermal cells and the structure of the stomatal apparatus of the reproductive structures, with those of the associated leaves, it has often been possible to determine which of the leaves belonged to the plants from which the fertile organs were derived.

Cuticle studies not only provided evidence that many of the mesozoic fern-like fronds belonged to gymnosperms, but they also assisted us in the reconstruction of the original plants.

As a result of this work we now know something of the general features of several types of the plants belonging to this group; we also know that there were many other allied forms whose reproductive structures are still unknown and which await further investigation.

Caytoniales

This was the first group to be reconstructed. In 1911 the writer discovered fruit-like structures and isolated seeds, which, on good cuticular evidence, belonged to the plant whose leaves had long been known as *Sagenopteris*, and thought to belong to a plant allied to the modern *Marsilea*. Later, the pollen-bearing structures were discovered and the material was described in 1925, when the group named the Caytoniales was proposed. Professor Harris has made further contributions to our knowledge of these plants, and he showed that the pollen reached the micropyles of the ovules as in typical gymnosperms. Since an account of this group has already been published in this journal (Harris, 1951), further description is unnecessary.

Peltaspermaceae

Antevs (1914) published an account of the cuticular structure of a fern-like leaf named by Schimper *Lepidopteris ottonis* and of the microsporophylls associated with it in Sweden. Both fertile and foliar structures had a stout cuticle with similar sunken stomata surrounded by a ring of subsidiary cells. The fertile organs were divided into several segments bearing elliptical pollen sacs, and had previously been named *Antholithus zeilleri*. Antevs concluded that *Lepidopteris* was really a seed plant and not a fern. This view was confirmed by Harris (1932) who found fine specimens of both these forms occurring together in East Greenland. With them he also found detached seeds and circular structures, which he called cupular discs, to which the seeds had probably been attached. The epidermal structure of the discs showed that they belonged to the *Lepidopteris* plant. About the same time Hamshaw Thomas (1933) discovered in the Molteno (Triassic) beds of Natal, South Africa, fronds referable to *Lepidopteris* (Fig. 3A) with a fertile axis accompanying them. This axis bore spirally arranged branches

terminating in peltate heads to which several seeds were attached on the lower side (Fig. 1) in the manner suggested by Harris. The peltate heads and the fronds showed the cuticular structure characteristic of the other species of *Lepidopteris*. Thus on the evidence from three widely separated regions this very fern-like frond was shown to be a gymnosperm and not a fern.

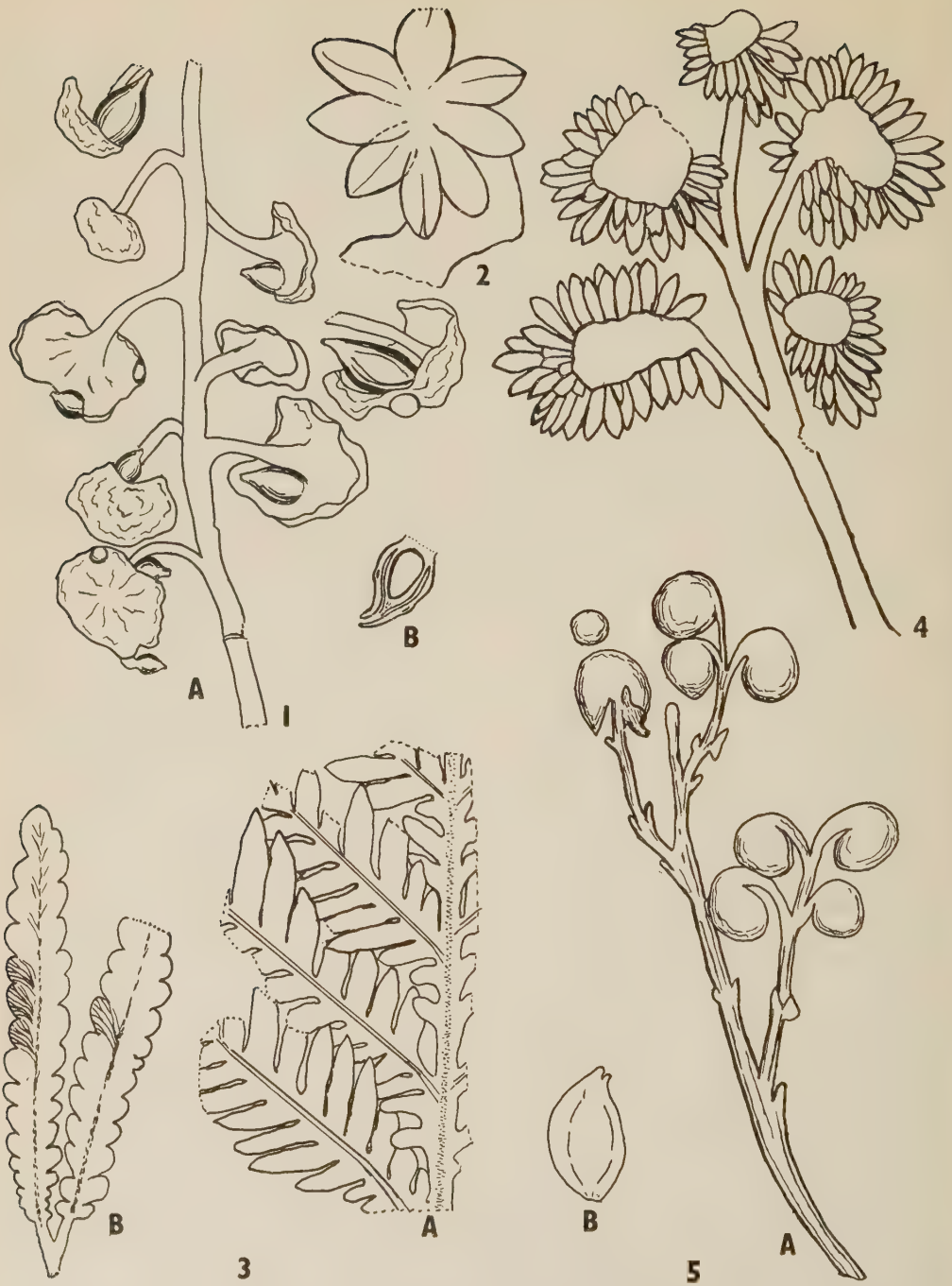
The stems of these plants are unknown. The fronds were from 8 to 30 cm long, bipinnate, with pinnules on the rachis between the pinnae. The stout rachis had a close series of blister-like swellings on its surface giving a scale-like appearance, these are not so conspicuous on the South African form. The closely set pinnules were linear lanceolate and attached by a broad base, they had a well marked midrib and forking secondary veins. Their upper and lower cuticles were thick, the stomata were chiefly on the lower epidermis. The pollen was produced on distinct male inflorescences[1] having a stout main axis from which short lateral branches were produced in one plane. The branches bore a considerable number of pollen sacs, each about 2 mm long and 1 mm broad, dehiscing by a longitudinal slit (Fig. 2). The oval pollen grains had a single longitudinal furrow. The seed-bearing axis was slender and several centimetres long, it differed from the pollen-bearing structure in the spiral arrangement of its branches. The peltate heads of these were 5 mm or more in diameter and bore a number of seeds near the margin on the lower side. The evidence of the southern form suggests that only one or two seeds on each disc reached maturity. Harris found that the seeds had a curved micropyle (Fig. 1B) and that the integument was free from the nucellus nearly to the base. The nucellus formed a short beak-like structure extending into the base of the micropyle, and probably breaking down to form a pollen chamber in which many pollen grains were observed.

1. The term inflorescence is used here in preference to the designation sporophyll. The fertile structures described in this paper are reproductive shoots bearing branches of limited growth, and thus come within the definition of an inflorescence when considered objectively. In typological morphology they may be sporophylls, but the concepts of typology are not really applicable to evolutionary morphology, and there is no evidence that the objects in question were parts of a foliar structure.

Corystospermaceae

The fossil plants placed in this group have as yet been found only in the Southern Hemisphere. Fronds and fertile structures occur in South Africa, Australia and Argentina; similar fronds occur in India, but the reproductive structures have still to be found. The Triassic plant beds in the regions mentioned contain many fronds in which the rachis forks into two equal halves, as in some of the Palaeozoic pteridosperms. They show considerable variety in form and have been assigned to four or five different genera. The more fern-like were formerly placed in the genus *Thinnfeldia*, a European type named by Ettingshausen in 1852, but they can be distinguished from the species of this genus by their cuticular structure. For this and other reasons Gothan (1912) named them *Dicroidium* (Fig. 3B). These leaves together with *Xylopteris*, Frenguelli (*Stenopteris*, Sap.), and other allied types occur abundantly in a plant bed in the Drakensberg in Natal, South Africa, and with them are found many pollen-bearing and seed-bearing structures as well as isolated gymnospermous seeds. The cuticular characters of the fertile structures agree closely with those of the leaves so it is highly probable that they were all derived from plants of the same group which Hamshaw Thomas (1933) named the Corystospermaceae.

The male inflorescences (Fig. 4), placed in the organ genus *Pteruchus*, were similar in general form to the palaeozoic *Crossotheca*. They had a central axis 3-4 cm long bearing short lateral branches generally arising in one plane but sometimes with a spiral arrangement. Each branch terminated in an elliptical or peltate head bearing, on the under side, a large number of lanceolate sporangia 1·5-3 mm long. The pollen, which may have been produced in two loculi, was probably shed through a terminal pore; the grains have



FIGS. 1-5 — Fig. 1. (*A*) *Lepidopteris natalensis*, seed-bearing structure. × 3·5. (*B*) *L. ottonis*, section of a seed, as reconstructed by Harris. × 2. Fig. 2. *Lepidopteris ottonis* (*Antholithus zeilleri*), group of microsporangia from photo by Antevs. Probably several more sporangia were originally present. × 7. Fig. 3. (*A*) Part of a frond of *Lepidopteris natalensis*. Natural size. (*B*) Frond of *Dicrodium odontopteroides*. Natural size. Fig. 4. *Pteruchus africanus*. × 3·5. Fig. 5. (*A*) *Umkomasia macleani*. × 2. (*B*) Isolated *Corystosperm* seed. × 3·5.

a characteristic form with two opposite bladders or wings symmetrically placed. Eight species of *Pteruchus* were recognized by their form and cuticular structure.

We have some evidence that the associated seed-bearing structures were produced on a main axis rather than on a frond, and most probably the pollen-bearing organs arose in the same manner. These female inflorescences (Fig. 5A) were slender branching structures, 3-4 cm long and often bore bracts and bracteoles. The lateral branches were usually produced in one plane, but one form showed a spiral arrangement; they were sometimes forked at the apex, and they produced other slender branches in an opposite or alternate manner. Each branch was recurved at its tip and bore a cupule containing a single seed. When young, only the curved micropyle of the ovule projected laterally from the cupule, but as the seed developed it emerged more and more from the cupule. While all of the same general type, the specimens collected in Natal could be separated into eleven species which were grouped in three genera, distinguishable by their general structure, the shape of the cupules and the cuticle structure of the cupules and axes. The commonest genus, *Pilophorosperma*, had its cupules lined with hairs produced from the epidermal cells of the inner surface; in some species the hairs were cuticularized and still remain, while in other species only the bases of the hairs are seen. Some of the cupules appear to have been divided into two lobes; their margins are generally entire. Many isolated seeds were found associated with the other remains, they are easily recognizable by a short-curved bifid micropyle (Fig. 5B), since these seeds show several distinct shapes and sizes it would appear that they were derived from different species. Their testas were smooth and hard, with a thick cuticle. Maceration showed little trace of a nucellar membrane except at the bottom of the micropyle where a small cylindrical cutinized structure was found. This structure seems to have been a nucellar beak, forming a pollen chamber, it was packed with the winged pollen grains like those produced in *Pteruchus*.

There can be little doubt that the reproductive organs just described came from the same plants which bore the forked fern-like leaves that occur with them. But we are not yet in a position to say which of the different fronds, male or seed-bearing inflorescences should be linked together.

While leaves of the *Dicroidium* and allied types are common in the Triassic beds of Australia, only a few reproductive structures have yet been described. Both Walkom (1917, 1925) and Jones & Jersey (1947) have described both pollen-bearing and seed-bearing structures, which appear to be closely similar to the species from South Africa.

The Triassic rocks of Argentina contain large, forking, bipinnate fronds previously known as *Thinnfeldia feistmanteli*, these were found by Frenguelli (1944) to be associated with slender forking axes whose ultimate branches terminated in a pair of small recurved cupules, like some of those of the South African corystosperms. The cupules show three equal marginal segments, but the ovules or seeds are not clearly seen. The same beds also contained pollen-bearing structures, which were named *Pterorachis*; they forked into two equal halves which bore fertile branches produced in one plane. These short branches bore a cylindrical mass of small elongated sporangia resembling those of *Pteruchus*. Although these structures do not seem to have furnished preparations of spores or cuticles, it seems highly probable that this plant, which Frenguelli named *Zuberia*, represents another distinct member of the Corystospermaceae.

Glossopteris

This has long been thought to be a fern leaf with reticulate venation. It is mainly represented by spceies of late Palaeozoic age but it also occurs in the Permian and Triassic rocks of the Southern Hemisphere. Plumstead (1952) has recently described fertile specimens from South Africa of Middle Ecca age (Lower Permian) which show that it cannot be a fern and is probably a seed plant, though the exact nature of the reproductive structures is still very obscure. They do

not resemble the fertile organs of any known type, and until other specimens are found in a mummified condition their interpretation must be somewhat speculative. The fertile leaves do not differ from the normal vegetative forms, but produced a short pedicel from the petiole, or the lower part of the midrib, which terminated in a compact ellipsoidal or fusiform group of bodies apparently enclosed in a bivalve cupular structure (Fig. 6). In the original descriptions these bodies were called sacs and were believed to have been produced on the inner surface of one half of the cupule. It is not known whether they were pollen sacs or seeds, but Mrs. Plumstead favoured the latter interpretation; in surface view they show as a close group of hexagonal heads, their sharp outlines suggesting that they possessed much hard tissue, and some of the specimens give the impression that they were 3-4 times as long as broad. Two genera, called *Scutum* and *Lanceolatus*, with several species, were distinguished; there can be little doubt that these are identical in nature with the problematical fossils previously described as *Ottokaria*. An active search for material in a better state of preservation is urgently needed, but the specimens now known, together with the discovery by Sahni (1923) that one species of *Glossopteris* had a resistant cuticle, make it fairly certain that this group must be included among the pteridosperms.

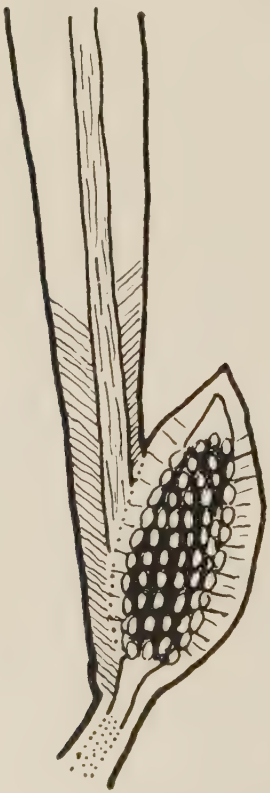


FIG. 6 — *Scutum dutoitides* on leaf of *Glossopteris indica* (*from* Plumstead, 1952). Natural size.

Leaves Referable to the Pteridosperms whose Fertile Organs are Unknown

The knowledge summarized above shows that several types of frond, fern-like in appearance but with resistant cuticles, belonged to seed plants. Certain palaeozoic genera such as *Callipteris*, *Mariopteris* and *Neuropteris* also had resistant cuticles, and all the evidence suggests that mesozoic ferns can be distinguished from the seed plants by the nature of their cuticular structure.

The first of the fern-like fronds (Fig. 7) with a thick gymnospermous cuticle to be described was named *Pachypteris* (Thomas 1954) by Brongniart in 1828, and since that time many other similar types have been recognized. In a recent survey of the fronds referable to the *Thinnfeldia* alliance, Frenguelli (1943) has recognized seventeen genera, but in addition to these there are probably some four or five other distinct forms. Most of these genera include a number of species. These different leaf-types are, however, based mainly on shape and venation, and might not deserve generic rank in a natural classification. On the other hand some of the species that have been placed in a single large genus like *Thinnfeldia* may prove to have been derived from plants that are generically distinct when their fertile parts are discovered. Full descriptions of all the leaves probably derived from pteridospermous plants cannot be given in this survey, but some brief notes may be useful.

FIG. 7 — Part of a frond of *Pachypteris lanceolata*. ×8/6.

The epidermal structure has been investigated in some species of the following genera, and has been found to be of a gymnospermous type: *Pachypteris* Brongn. *Thinnfeldia* Ettingsh., *Cycadopteris* Zigno, *Lepidopteris* Schimper, *Dicroidium* Gothan, *Amdrupia* Harris, *Pachydermophyllum* Thomas & Bose, *Xylopteris* Frenguelli, *Stenopteris* Saporta, and of *Sphenopteris* Brongn. All but two of these genera have thick resistant cuticles, with few stomata on the upper epidermis; the stomata were usually sunk below the leaf surface and the guard cells surrounded by a ring of over-arching subsidiary cells. In *Pachypteris* and *Cycadopteris* the stomata were grouped very closely in bands, as in some conifers.

Fronds with a forked rachis, as often found in the Lower Carboniferous rocks, are common in the Southern Hemisphere, this character occurs in *Dicroidium*, *Xylopteris*, *Zuberia*, *Johnstonia*, *Diplasiophyllum* Frenguelli (= *Danaeopsis*, pars), and *Dicroidiopsis* Frenguelli. *Dichopteris* seems to be the only form from the Northern Hemisphere (apart from India where many of the southern genera occur) which shows this character, unless we include *Ptilozamites* Nath. among the likely pteridosperms.

The cuticular structure of several genera are still unknown, but since they resemble those mentioned above in form and venation it seems highly probable that they belong to the same class. There are also certain mesozoic forms whose fronds are perhaps nearer to the cycads than to the ferns in general form, but which are quite distinct from the Bennettitales and Cycads in their cuticular characters. Among these are *Ptilozamites*, *Ctenopteris* Brongn. and *Ctenis* Lind. & Hutt. Their reproductive structures are unknown and since it is possible to regard their fronds as evolved from the *Odontopteris* type of the Upper Palaeozoic, they may well be related to the pteridosperms. The genus *Rienitsia* Walkom may also be regarded as having affinities with the pteridosperms. Its leaves are somewhat fern-like while its cuticles, described by Jones & Jersey (1947, p. 41), resemble those of *Ctenis*. Its microsporangia appear to have been borne on a leaf-like lamina.

Remarks and Comparisons

We have as yet no knowledge of the habit or of the stem structure of the plants mentioned above. It is not improbable that the petrified stems named *Rhexoxylon* belonged to some pteridosperm from the Southern Hemisphere, since its structure is more like that of some of the Permian medullosas than any other known type. But there is no evidence to connect this form with any foliar or reproductive structure. The great abundance of leaves of one species found in some localities suggests that they were borne on plants that were trees or shrubs, and probably had deciduous leaves.

Very few of the mesozoic species had fronds which approach those of the Upper Palaeozoic pteridosperms in size. Many of them were small, with a simple pinnate or even an entire lamina. The pinnules were commonly attached by a broad base, and the odontopteroid type of venation is usual. Taken as a whole they suggest

the evolution of a smaller, more compact leaf, when compared with the highly dissected forms from the earlier rocks. Reticulate venation sometimes occurs, but apart from *Glossopteris*, and the leaf of *Caytonia*, little approach to the angiospermous type of leaf is to be seen. The thick cuticles and sunken guard cell of many of the forms may be the result of natural selection brought about by the dry climates of Permian and early Triassic times.

It seems certain that the seeds and pollen of the mesozoic pteridosperms were borne on small branch systems or inflorescences, some of these are branched in one plane only and may be regarded as fertile fronds without a lamina. Although in some of the palaeozoic types the seeds and pollen were produced on the ultimate branches of fertile fronds with a well developed lamina, it is by no means certain that all the genera bore their fertile branches in this way. Some of the latter may have been produced directly on the stems, as is seen in the Corystospermaceae.

The fact that several of the mesozoic inflorescences were branched in one plane while others had a spiral arrangement of branches, is a point of some interest since it is seen in both the pollen- and seed-bearing structures. It suggests that the spiral mode of branching in the fertile parts may have arisen from a system in which the branching resembled that of a photosynthetic frond. This removes one of the objections to the view that the angiosperms may have evolved from one or more types of pteridosperm.

The cupules of the corystosperms serve as a link between the type of cupule seen in the palaeozoic pteridosperms and that found in *Caytonia*. The winged pollen of *Pteruchus* and *Caytonianthus* must be considered as adapted for upwards flotation in a pendant receptive structure, as in the recent members of the Coniferae.

Although not closely similar to any of the known members of the Bennettitales and Cycadales, the forms described above suggest possible ways in which the fertile structures in these two groups may have evolved from pteridospermous organs (Thomas, 1950).

The occurrence of pteridosperms in many parts of the world prior to the Cretaceous period would lead one to expect that this group should be represented in the flora of the present day. Among the modern ferns, conifers, and cycads are types closely similar to certain mesozoic genera; the Ginkgoales are still represented by one species. The Bennettitales, already highly specialized by Lower Cretaceous times, are extinct. But the relatively unspecialized pteridosperms seem to have died out also, unless *Gnetum* and *Welwitschia* belong to this group. This may be because the angiosperms, a race of plants with a better equipment for competition, needed the same habitats and displaced the pteridosperms from them. Future work will probably show that the angiosperms themselves have evolved from some members of the pteridosperm alliance, at present unknown; a thorough search should be intensified in India and the Southern Hemisphere for such forms.

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HILDENBRANDIA IN AMERICA

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In 1845, the genus *Hildenbrandia* was established by Nardo to characterize and differentiate a marine plant of European waters which formed minute red incrustations on submerged rocks. This plant as *H. prototypus* Nardo has been recognized as of wide distribution, and plants conforming to the description of Nardo have been reported from various marine habitats in this country. A second marine species, described as *H. crouani* by J. Agard, has been reported from various European habitats.

At an early date a fresh water species of the genus *Hildenbrandia* was recognized and came to be designated as *H. rivularis* (Liebm.) Brèb. This species was found to be widely distributed in Europe. In the United States this fresh water species was reported by Wolle in 1878, and on the basis of this report it was included in the 1933 edition of Smith's "Freshwater Algae of the United States". Since Wolle did not list specific collection points and some doubts had arisen regarding the habitat, and particularly since the plant had not been reported by other American botanists in the long period of intervening years, the genus *Hildenbrandia* was omitted from the 1950 revision of Smith's volume.

In a general appraisal it would appear, therefore, that up to the present time the genus *Hildenbrandia* has been accorded authentic recognition in America only as represented by the marine red alga, *Hildenbrandia prototypus* Nardo. This plant has had the adequate attention of Taylor.

Description of American Fresh Water *Hildenbrandia*

On November 18, 1954, the writer collected plants in a swift-flowing stream at New Braunfels, Texas, which in a

general way appeared to conform to descriptions of the *Hildenbrandia rivularis* of Europe. Since the collection seemingly warranted the restoration of the plant to the list of fresh water red algae of this country, it was considered appropriate to study the material in some detail and thus to characterize it for inclusion in our flora.

The plants as collected had developed as orange-red or blood-red more or less discoid crusts, often confluent or overlain, in masses extending to the order of two centimeters in length or diameter. The plants were not obvious from an exposed position above the surface of the stream, but were rather sharply restricted to well-shaded surfaces of submerged rocks. Upon over-turning such structures the plants became very conspicuous as glistening vermilion blotches which became blood-red upon drying. In some of the European habitats the plant was reported as having been accompanied by other lithophyllic algae. In contrast, the American plants of the specified habitat for the most part were free from other algae. On the more exposed rock surfaces, however, there had developed extensive growths of the chantransial stages of several species of red algae, with an occasional incidence of tufted blue-green algae. The respective zones, which appeared to be conditioned by light, were at times somewhat contiguous, and in material from such regions it was common to find thalli of *Hildenbrandia* with apparent extrusions of upright chantransoid filaments. These filaments, however, uniformly lacked the internal cellular characteristics of the *Hildenbrandia* and without exception were identified with chantransial plants dominating the exposed zone. In no instance was there found any development of extraneous filaments from the crustose thallus of the *Hildenbrandia*. The observations thus were in accord with those of Fritsch respecting European material of *H. rivularis*, and tended to sustain his viewpoint that such filaments, drawn by Budde (1926) and reproduced by Smith (1933) did not originate from the *Hildenbrandia* thallus.

Scrapings from the fresh vermilion blotches, when placed upon a microscope slide and examined under the low power of a compound microscope presented pinkish or cerise crusts in two conformations. Viewed under high power from a point above the outer surface of their original position their appearance was that of a honeycomb of cell walls with homogeneous pink coloration. Their appearance has been indicated in Fig. 1. This effect was found to be attributable to the lenticular nature of the exterior walls capping in a uniform plane the stratum of vertical contiguous filaments, as shown in Fig. 2.

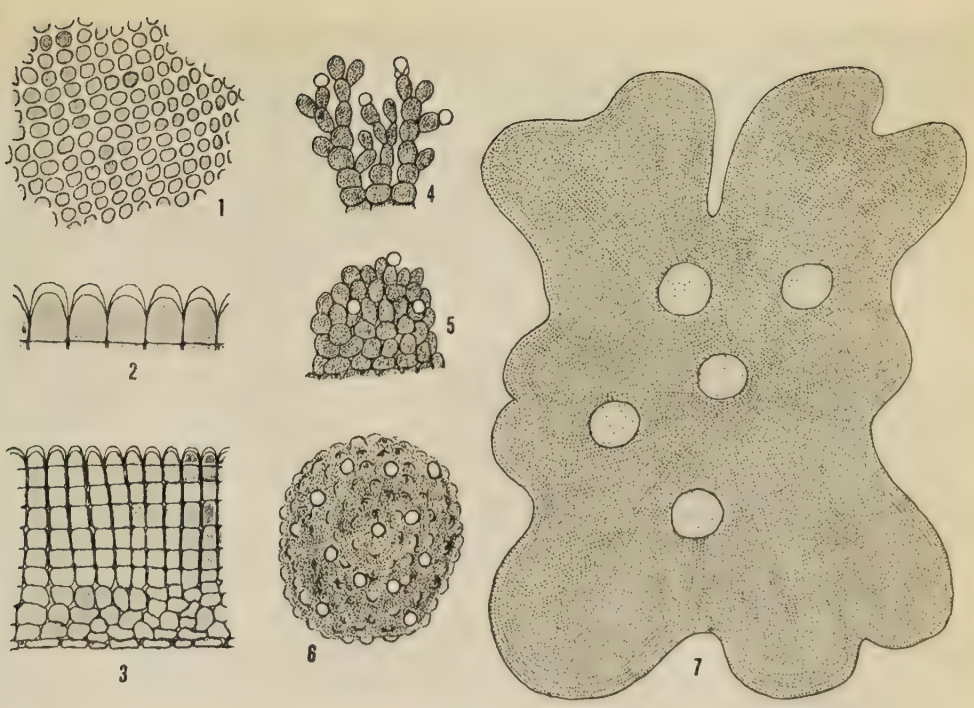
Viewed under high power from a point above a segment of the crust placed on its side, the structure was observed as comprised of contiguous and compacted filaments of cells in palisade arrangement which surmounted a basal matrix of unoriented cells, the layer adherent to the rock surfaces. This general view is shown in Fig. 3.

Although the thalli in general were discoidal, when unrestricted locally, in numerous instances both the peripheral and the sub-peripheral growth was so irregular as to suggest active regeneration. In some of the older thalli circular areas of greater transparency disclosed the incidence of tetrasporic conceptacles.

Viewed under oil immersion the plastids were noted as laminate in the parietal position, one in each cell. With respect to intercellular connections no passage ways were observed, but the protoplasm was noted as contiguous to apposed cell walls at the same focal point, thereby suggesting connection.

The cells of the filaments which comprised the palisade portion of the thallus were of the order of 8 μ in diameter. The thickness of the crusts was found to range from 35 to 150 μ.

An extended search was made for the monospore-like bodies reported by Skuja (1938) for the *H. rivularis* of Europe. On the adult structures these were lacking or very rare; on the young growth near the tips of advancing thalli and on regenerating structures developing locally on old thalli, terminal cells of reduced pigmentation were cut off as globose thin-walled spores. These structures are

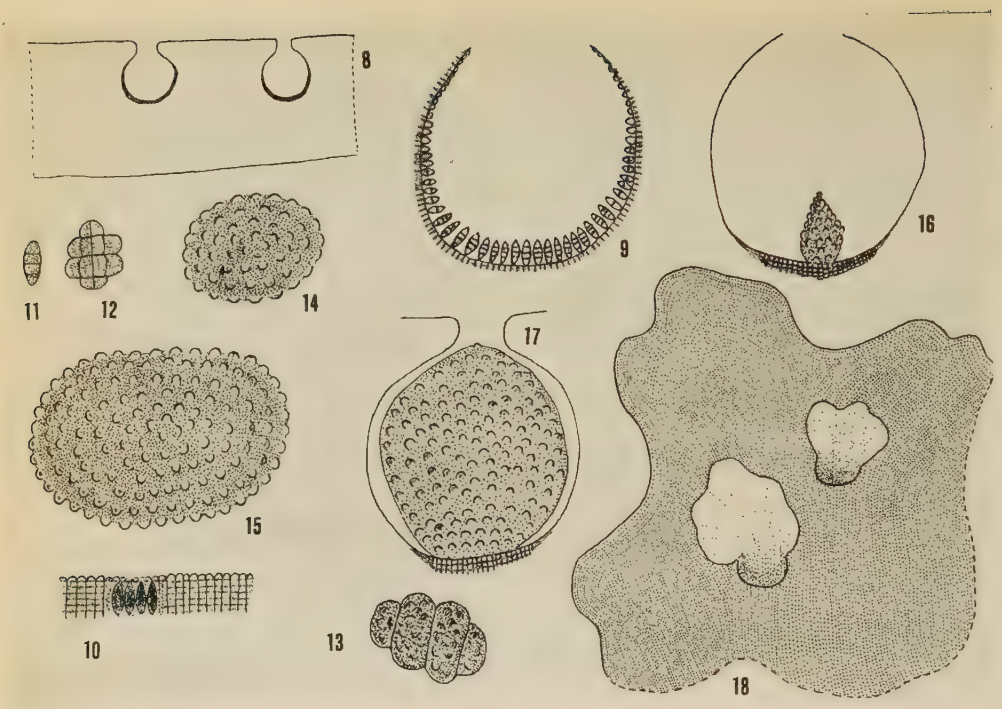


FIGS. 1-7 — Fig. 1. Surface view of thallus. Fig. 2. Sectional view of capitate cells. Fig. 3. Sectional view of palisade tissue. Figs. 4, 5. Terminal new growth of thallus, showing monospores. Fig. 6. Gemma-like thallus developed from tetraspore, showing monospores. Fig. 7. Thallus, showing conceptacles. Approximate magnifications: Figs. 1-6. × 100; Fig. 7. × 15.

shown in Figs. 4 and 5. The monospores, however, were later found to be borne in great profusion on the gemma-like thalli which developed from the tetraspores, as shown in Fig. 6.

The tetrasporic conceptacles were found during the search for monospores, since in some instances these globose wide-mouthed cavities were appreciably more translucent than the general matrix, as has been indicated in Fig. 7. On the other hand, it was noted also that in the older conceptacles a progressive series of stages of regeneration was represented. Regeneration was initiated at a point at the base of the conceptacle crater, as shown in Fig. 16. At this time some tetraspores were being produced along the upper walls and released into the cavity. Regeneration continued and the conceptacle cavity became filled with a globose thallus, as shown in Fig. 17. The growth then continued and resulted in the disruption of the conceptacle tissue around the opening and a subsequent radiate overlay of the old thallus, as shown in Fig. 18.

The tetraspores were cut off apically as spindle-shaped four-celled structures oriented in the manner of the palisade filaments and similarly pigmented. The production of tetraspores as evidenced by an examination of the conceptacle walls had begun at the crater base and had proceeded radialy upward from this point, as shown in Fig. 9. There was no evidence of tetrasporangial stalks or of sterile paraphyses. In a few instances tetraspores were found imbedded in thallus tissue having no obvious relation to a conceptacle, as shown in Fig. 10. Discharged tetraspores were present within numerous conceptacle cavities, but only in small numbers.



FIGS. 8-18 — Fig. 8. Section of thallus showing conceptacles. Fig. 9, Section of conceptacle showing tetraspores. Fig. 10. Section of thallus showing tetraspores. Figs. 11-15. Stages in the germination of tetraspores. Figs. 16, 17. Stages in regeneration of thallus tissue within old conceptacles. Fig. 18. Thallus with regenerative thalli from old conceptacles. Approximate magnifications: Figs. 8, 18. × 15; Figs. 9, 16, 17. × 80; and others × 100.

By virtue of a fortunate collection of material at some distance downstream from the habitat of the above described plants it was found that released tetraspores in great abundance had lodged among the gelatinous filaments of tufted chantransial plants of other genera of fresh water red algae. Here they were present in stages affording a developmental series ranging from four cells to an estimated two hundred cells, as indicated in Figs. 11-15, also in Fig. 6. The first cell divisions which took place in the germination of the tetraspores were in a plane parallel with the axis, and even the largest of the structures observed had a somewhat flattened though bilaterally symmetrical thallus. The cells of these structures were radialy oriented, but there was no palisade arrangement. Some of the older thalli bore monospores in profusion, identical with those observed on the younger portions of the crustose thalli (cf. Figs. 4-6).

Discussion

With respect to the life history of the plant, a major point for further research is the matter of the sexual organs, which were not found in the material collected. A second point of interest is the determination of the fate of the gemma-like structures which develop from the tetraspores upon germination. Correlated with these points is the nature of the seasonal changes which take place to comprise the life history of the plant.

With respect to the distribution of the plant in this country one may anticipate that numerous habitats will be found in which it is indigenous. Two features

well may have contributed to its seeming rarity: (1) its best growth takes place on the under side of stones in swift water and it is not obvious from a casual examination of the stream, and (2) its general appearance is that of a vermilion blotch which easily could be mistaken for iron rust, for diatoms, or for bacteria.

With respect to the identification of the plant there appears to be no reason at this time for anticipating that the American plant is distinct from or other than the *H. rivularis* (Liebm.) Brèb. of Europe. It is recognized that an adequate description scarcely could be expected from the material of a single collection, but it has been the thought that even this inadequate beginning might contribute to an interest in the genus and facilitate further collections and study. In some recent publications the plant has been designated as *H. rivularis* (Liebm.) J. Ag.

Summary

In 1845, the genus *Hildenbrandia* was established by Nardo to accommodate a marine red alga of European waters and subsequently, a freshwater species was found to be widely distributed on the continent. This paper reports the discovery of a freshwater species of *Hildenbrandia* in the United States. Several important aspects of the life history remain to be determined, but in general the plants appear to conform to descriptions of the European freshwater species *H. rivularis* (Liebm.) Brèb. and in consequence the American plants are so designated at this time. Several features rather unusual in freshwater red algae — the restriction to weak light, the red coloration, the development of a palisade tissue, the occurrence of tetraspores in conceptacles — make this plant a most attractive addition to our flora.

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SOME REMARKS ON MYRMECOPHYTES

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Introduction

During work on the pollination of some flowers by carpenter bees (*Xylocopa*) in Java the author was struck by the relation of both with ants. The floral biological observations have been published elsewhere[1]. Here I wish to draw attention to known relations of ants with tropical plants. This field deserves to be taken out of the cabinet of biological curiosities and disputed romantics created by earlier travellers in the tropics.

In the tropics the abundance of ants and their general influence, even on the human household, suggest some relationships. In a sense the role of ants might be compared and put at par with other physiological and biological factors such as high temperature and special pollinators.

On a previous occasion (1953) I pointed out that the biology of tropical plants can be understood fully only if the climate factor is considered along with others equally significant in tropical life such as biotic influences. It is a common attitude to regard ant relations as incidental and not belonging to the normal scheme of life. This is understandable when we consider that botany originated in temperate regions where ants are less influential. However, the old and narrow Europe-centric concepts should be discarded and tropical plants should be accepted as fundamental.

It is not sufficient merely to record incidental finds on plant-ant relations; the problem as a matter of fact needs to be intensively investigated. Moreover, not only positive but also negative results are of interest because quite often they reveal inter-relationships which otherwise remain obscure.

To maintain uniformity and to avoid ambiguity a strict use of definite and specific terms is proposed. Most of the terms included in this discussion were coined before, mostly by Warburg (1892). He employed the term " myrmecophyte " for all plants that have regular relations with ants. I wish to reserve the general term " myrmecophytism " for the bondage, referring neither to the plant nor the ants. The term " myrmecophily " may however be retained for the flower, in analogy with comparable terms such as " ornithophily ". Preferably all terms should begin with " myrmeco ", and the second half should indicate the partner in the relation. But this can lead to some confusion. The relation between the first and the second half can be object-subject or subject-object; for instance, the term " myrmecophoby " could mean fear of ants instead of fearful to ants. Object-subject relations, however, have, as far as I know, no generally acknowledged means of expression in scientific terminology.

The categories of myrmecophytism used here are: (i) myrmecophily, pollination of flowers by ants; (ii) myrmecotrophy, feeding of ants; (iii) myrmecodomy, housing of ants; (iv) myrmecophylaxis, protection by ants; (v) myrmecophoby, deterrence of ants; (vi) myrmecochory, dispersal of seeds by ants.

The present study is in many respects an extension of the one by Warburg (1892), who gave a good review of cases known up to his time. This communication is somewhat sketchy being based on field notes which were lost during the war and revolution in Java. Ants are considered in this article more or less in a general way. This approach is not free from risk, and it certainly needs to be supplemented by a specific study of the ants.

1. Proc. Kon. Ned. Akad. Wet. Amsterdam. 1954.

The rejection of the myrmecophytic nature of a plant on the ground that "the ants" are indifferent to its adaptive structures is often unjustified because the specific, natural ants may be lacking. In the botanical garden of Bandung and Bogor true myrmecophytes, like *Cecropia* and the *Acacia* species (both imported), and even the true Indonesian myrmecophytes, like *Myrmecodia* and *Endospermum moluccanum* (T & B) Becc. are entirely or partly neglected. This happens even after transplantation along with true "symbiotic" ants (cf. Rant, 1934, on *Endospermum*). Often one ubiquitous local species drives away the true inhabitants without taking over their role. In *Endospermum moluccanum* the Javanese ants cannot reach the interior of the typically swollen branches. The true inhabitant in Ambon (*Camponotus quadriceps* F. Smith) gnaws holes to take away the pith. Other ants seem to lack the necessary instincts. However, the tree thrives well without ants.

Zoologists (*see* Wheeler, 1942) on the other hand are sometimes inclined to pay attention to the ants only and consider plants as non-adaptive, passive subjects.

It seems unjustified to demand proof of advantage to the plant in order to admit an otherwise obvious unity of plants and ants. Even in the closest adaptations between flowers and special pollinators the advantage to either side is often questionable. The advantage may moreover be physiological and ordinarily undecipherable.

Myrmecophily

Ants are not fit to act as pollinators because they cannot fly from flower to flower and are not disposed to carry pollen. Therefore, their significance in pollination has to be accepted with reserve.

Quite often the difference between an incidental visitor and a regular pollinator is overlooked, as for instance in the uncritical report by Migliorato (1910) from Italy on *Rohdea japonica*.

In recent years a few isolated cases have been published from northern countries. *Seseli libanotis* L. which is normally pollinated by flies and beetles, seems to set fruit in Denmark after visits by *Lasius niger* L. (Hagerup, 1943). Dahl & Hadac (1940) considered ants as regular pollinators of the seashore plant, *Glaux maritima*, L., Norway. Hagerup (1932) believes that under extreme conditions (for instance in the dry climate of Timbuctoo) some flowers may be pollinated by ants. He, however, does not mention a single case where this has actually been observed. The data cited by Winkler (1907) on cauliflorous flowers possibly pollinated by ants also seem to rest on mere assumption. His supposition concerning *Theobroma cacao* has since been refuted, and the only instance where he makes a positive statement to this effect lacks reference to observations.

Myrmecotrophy

This was one of the most discussed topics of early tropical ecology. In the days of Delpino, Belt and Schimper the feeding of ants by plants was often romanticized. Sometimes to every extrafloral nectary a sign of "myrmecophily" was ascribed. It should, however, be remarked that Schimper himself was somewhat skeptical of this approach.

In later years Frey-Wyssling (1935) and others assumed an ultra-critical attitude, based in my opinion on a somewhat exaggerated fear of teleology. In Frey-Wyssling's opinion one has to choose between ecological (so-called teleological) and physiological theories. He prefers the physiological explanation given by Liebig that extrafloral nectaries secrete superfluous carbohydrates in growing organs.

There may be a good deal of truth in this "Saftventil" hypothesis, but many questions remain unanswered by it. For example: Why are such intricate organs so often found on tropical leaves? Why is there a considerable distance between "Saftventil" and the growing leaflets? Further, what about the old data of Schimper on *Cassia neglecta*? It is curious that Burck (1906), the romantic ecologist, was in one respect "plus physiologique que le physiologiste" as he tried to explain the floral nectaries on a physiological basis. He believed that their secretion served to draw water

from the anthers, thereby promoting dehiscence.

In my opinion it is not essential to choose between the two ways of thought. Of course, the extrafloral nectaries may be primarily physiological organs, but in some instances it is possible that they have acquired other functions.

If floral nectaries have a recognized (although secondary) function towards animals, why cannot some extrafloral nectaries, in the first place those near the flowers, have a similar role? There is moreover a transition from extrafloral to floral nectaries (nuptial extraflorals) and vice versa. Mention may be made of cases, included in the extensive discussion by Zimmermann (1932), where entire flowers have been transformed into nectaries near the base of normal flowers. They are best known in the papilionaceous genera *Phaseolus*, *Vigna*, *Dolichos*, *Centrosema* and *Canavalia*. I described the case of *Honckenya ficifolia* Willd. (Pijl, 1951) where even the lower vegetative parts bear nectaries consisting of transformed flowers. In Java these nectaries attract ants. Their ecological significance remains to be studied in the natural habitat.

In all the papilionaceous genera mentioned above, I found tropical species where nectaries regularly attracted ants to the specific area. This will be pointed out in later paragraphs under "Myrmecophylaxis" as an instance of the ecological function of prevention of robbing by the pollinators.

When in some smaller flowered and in some extra-tropical *Phaseolus* species the nectaries do not fulfil the function of myrmecophylaxy, this may be considered as secondary, the organs being relics of the original tropical condition. This may even apply to the much discussed stipular nectaries of *Vicia* species in Europe.

Generally the initial considerations themselves of extrafloral nectaries have been wrong. The peculiarity is not the abundance of ants in the tropics but rather their scarcity in the temperate regions. Physiological reasons alone may not account for this.

Judging from the waning animal life in the northern countries, it is no wonder that the relations between plants and ants are also not represented in normal proportions. Europe-centred researches on the subject, like the one by Springensguth (1934, 1935), cannot be considered as having a general importance. When a non-temperate plant, like *Ricinus*, is investigated in northern Europe (*see* Springensguth, 1935) the conclusion is: "The extrafloral nectaries have no ecological significance for the plant." Springensguth should see *Ricinus* and other plants with nectaries near the flowers in the tropics and count the swarms of ants on *Thunbergia grandiflora* there. An error of the same kind can, however, be committed even in the tropics if floralbiology is neglected. A case in point is that of Nieuwenhuis (1907) who rejects the ecological value of the gigantic extrafloral nectaries of *Poinsettia*.

Of course the great majority of foliar nectaries in the tropics have only the original significance, though they may attract insects. With many insects, not necessarily ants, a facultative bond has been developed. Peculiarly enough ants may even be rare on them. Wasps of all kinds have a remarkable aptitude for finding inconspicuous nectaries, and depend on them for their carbohydrate needs.

As in *Ricinus* nectaries, in some *Ipomoea* species also the calyx bears nectaries which attract wasps. Even the big *Vespa analis* sucks them. *Sceliphron violaceum* and Chrysididae are quite common there. Malvaceous leaves are an important source of food to such wasps in Java. *Hibiscus rosa sinensis* leaves are frequented by a small *Ropalidia*. In *Hibiscus radiatus* Cav. there is nectar secretion on the persisting calyx leaves around the young fruit after anthesis. Three *Eumenes* species and an *Apanteles* species were seen there. In *Urena lobata* L. this happens during anthesis. Do the cases of *Hibiscus* and *Urena* belong to the domain of physiology?

In the cyathia of *Euphorbia* and *Pedilanthus* the transition to entomophily by means of foliar nectaries is complete and no botanist can doubt their ecological nature.

In general the transformation of "physiological" nectaries to those with a more or less myrmecotrophous function may

be recognized in many ways. The location, dimensions, time of functioning and associated structures all throw light on this transition.

Further, I found a decided myrmecotrophic progression in the prolonged life of the floral nectaries of *Myrmecodia rumphii* Becc. — the Ambonese species so well known for its myrmecodomy. The fruit swells after fertilization and throws off the corolla. This swelling, however, concerns only the upper part containing the nectary. The seeds occupy but a small part of this young fruit. Through the apical pore great quantities of nectar flow for a long time.

The most obligatory myrmecotrophic nectaries are those of *Thunbergia grandiflora* Roxb., the carpenter bee flower par excellence, described as early as 1891 by Burck. But for his error in considering nectaries as food bodies, Burck's work may be considered fairly important in the field. The glands are small, cup-like trichomes, that are certainly no "Saftventile" near growing organs. When functioning they are white, the cups overflowing with nectar. After one or a few days they wither into black shapeless remnants. By a gradual shift of activity from the nodes of vegetative branches successively to the peduncles and the flower bracts they seem to guide the ants to the flowers.

In connection with the kind of food offered by plants in the form of food bodies, I only wish to point out that these organs seem to appeal only to special tastes of certain ants. Neither ant-Acacias nor *Cecropia* attracted ants in the Hortus in Bandung. That the bodies are not structures *de novo* and may be derived from hydathodes is morphologically interesting but has no bearing on their ecological value.

There are leaf nectaries, as in *Nepenthes*, of undoubted significance in the trapping of insects. Further no one questions the significance of myrmecochory in the life of many plants ever since Sernander (1906) published his monograph on the subject. He pointed out a possible connection of myrmocochory with myrmecotrophy as effective in procuring the presence of ants when the fruits are ripe. He mentions *Melampyrum pratense* and *Lamium album* in this connection. This point deserves closer attention in tropical countries.

Myrmecodomy

Ants as a rule do prefer a cover, not only for nesting but also more temporarily when feeding. In *Cassia alata* L. I observed that ants covered the secreting stipules with hairs from other plants, thus making secluded corners. Beginnings of structural adaptations of this kind, though more or less incidental in character, can be found in some *Albizzia* species in which the lower pinnae of the leaf are reduced in size and curved back over the nectary on the basal part of the rachis. In some species of the *Conchophyllum* group the peltate leaves are pressed close to the bark of a tree. This leads to incidental myrmecodomy. The pitcher leaves of *Dischidia rafflesiana* Wall. have a recognized physiological function. Ants (that play a role in the dispersal of the plant) often use these pitchers as a home, closing off the aperture. This impedes the normal function of water collecting but may result in more nutrients for the plant. It occurs regularly and might lead to some shift in functions — as happened perhaps in *Myrmecodia*.

That there is no sharp demarcation between obligate and facultative myrmecodomy can be seen in some *Acacia* species, also known as ant-plants. Several authors believe that the swollen thorns of many African *Acacia* species are not adaptations of the plant to ants but are galls formed by hymenopterous larvae. Skwarra (1913) reinvestigated the American species and found very specific inhabitants. Wheeler (1942), the well known ant-specialist, neglecting the other myrmecophytic characters of these *Acacia* species, tends to minimize their nature as myrmecophytes. Indeed the adaptation is not so simple as was thought, but this should not lead us to deny any reciprocal bond. The bond may be part of a biotic triangle — plant, larva, ant — each being equally essential.

In Bandung the thorns of a cultivated specimen of *Acacia spadicigera* Cham. & Schl. (an American species) were never inhabited. When, however, holes were

made in them, small black ants (unidentified species) gnawed away the pith and went to live in these thorns. They neglected the food bodies on the leaflets.

I found in Java an additional case of myrmecodomy in an indigenous species, *Acacia tomentosa* Willd. In a mixed teak forest north of Bandung most of the thorns of this tree were found inhabited by ants. Often these belonged to a species not found elsewhere and new to science. Just as in the African and the American species the tree is a part of the triangle. The larva of a microlepidopteron lives in the thorns when these are still soft and green. It hollows them out partly and leaves an exit hole, through which the ants gain admission to the central canals in the twigs, which acquire the typical brown lining.

In the case of *Endospermum* referred to above, the relation appears to be more obligatory. As Rant (1934) described, I also saw females of *Camponotus quadriceps* F. Smith bite their way through the peripheral cylinder of the branches to the pith. But even here where the swollen twigs betray the adaptive nature of myrmecodomy and where leaf nectaries offer large amounts of nectar, a third factor has some influence. I noted some preference in *Camponotus* to begin gnawing at small scars.

Myrmecophylaxis

On this point, indeed, unfounded generalizations have been launched by early writers. It is high time that the idea of general protection through attracted ants is given up. On the other hand, a specific protection can often be demonstrated. Kerner advocated the idea that sometimes ants on extrafloral nectaries near the flowers may serve to protect the flower against gnawing beetles.

Here I want to discuss more fully Burck's (1891) idea of " ant patrol " as protecting many tropical flowers from raids by carpenter-bees (*Xylocopa*). Later on a general skepticism against unproven and romantic ant stories arose and a preference for causal physiology became prevalent. Nieuwenhuis' (1907) criticism further influenced public opinion, so that the study by Burck and in fact the topic on the whole began to be disfavoured.

Having studied many flowers adapted to *Xylocopa* I can demonstrate the existence and specificity of the protection.

The tropical *Xylocopa* species are excellent pollinators but have a strong tendency towards robbing flowers. The flowers adapted to them have contrivances to exclude visits of smaller insects and this necessitates the use of effort by the carpenter-bees. It might induce them to use their exceedingly strong mouthparts in an illegitimate way, as indeed they do in the non-adapted flowers that nevertheless attract them.

All the genuine Xylocopa flowers, for example, *Costus speciosus* (Koen.) Sm., *Thunbergia grandiflora* Roxb., some *Ipomoea, Centrosema, Canavalia, Dolichos, Vigna, Phaseolus* and *Cassia* species, and even some orchids have some kind of specific protection against this raiding. Occasionally it is in the form of hard flower bases or hard bracts and often an ant guard near the base attracted by the nectaries.

The species of ants does not seem to count in the triangle of flower-ant-*Xylocopa*. Only once I found the equilibrium disturbed when the rather small flowers of *Dolichos lablab* L. had attracted specimens of the very large and ferocious ant *Oecophylla smaragdina* on the calyx. *Xylocopa* individuals trying to land flew off at once when perceiving the ants in alarm position.

Surely it is unduly simplifying the problem if we eliminate the question of myrmecophylaxis, as Frey-Wyssling (1935) does by stating that the ants on the nectaries are almost without exception harmless vegetarians without protective value. Malicious or not, the ants do deter *Xylocopa*.

When I made small holes in the flower tube of *Thunbergia grandiflora* and enticed some ants by means of sugar to enter the tube from the calxy, these few vegetarians were always sufficient to scare off the normal visitors, viz. *Xylocopa latipes*. From the buzz the bees made near the flower I recognized some element of fright or annoyance.

In a group of *Cassia bacillaris* L. shrubs which were frequented by *Xylocopa*, one

branch had flowers with aphids on the bases of petals and pistil. Some ants were visiting the aphids. Doubtless, these few flowers were avoided after being approached by the bees.

Ipomoea crassicaulis (*I. carnea* Jacq.) has as pollinators some smaller *Xylocopa* species like *X. caerulea* F. and *X. confusa* J.P. When ants are present in the neighbourhood, they visit the nectaries in the calyx, debouching in the corners between the lobes, so that nearly always a few specimens are present near the base. In Bogor the legitimate visitors against whom the protection seemed adequate, did not raid the flowers. Some specimens of the bigger *X. latipes* F. (not able to pass legitimately through the flower tubes) landed on the flowers. They walked down the outside of the tube. Because of their longer mouthparts they knew to evade the ants better than the normal pollinating *Xylocopa* could do. They inserted their maxillae some distance higher up, above the nectar room proper, in the broader part of the tube. On one flower quite a number of ants were for some reason walking on the tube base itself. The would-be raider (*X. latipes*) here came into contact with them. It produced the familiar buzzing sound and disappeared hastily, leaving the whole bush, which it had been raiding for some time.

This precise observation is more valuable than the critical but all too general report by Nieuwenhuis (1907) that in *Ipomoea crassicaulis* an ant guard is present, but that *Xylocopa* nevertheless bites holes in the flowers. I hesitate to accept that she actually saw some *Xylocopa* " bite " among a group of ants (there is moreover no " biting ", only a slitting by the pointed combined maxillae).

A safeguard against deviations of the legitimate pollinator may be ineffective or less effective against other insects. For this reason, however, it is not valueless. A protection is specific and competitive.

In an open field near Bandung *Ipomoea crassicaulis* was living without an ant guard, as apparently no suitable ants lived there. Here 100 per cent of the flowers were punctured by *X. latipes*. At some kilometres distance in a garden with many ants the same *Ipomoea* had well-manned guards of large and exceptionally mobile ants. I could ascertain there that a *X. latipes* tested some flowers, made a half-hearted attempt at robbery and disappeared. The percentage of punctured flowers at the end of the day was very low here, being about 1 per cent.

The importance of special protection is demonstrated by a comparison between *Thunbergia grandiflora* (which is never robbed) and sister species without protection and destined for bees other than *Xylocopa*. Burck (1906) described *T. moore* as 100 per cent punctured and I saw the same in *T. erecta* T. Anders. In gardens in Java *T. grandifolia* often grows along with *Arrabidea* (*Bignonia*) *magnifica* Sprague. whose flowers are almost the same when seen from the front, but are much narrower in the tube and are unprotected so that they are 100 per cent robbed.

Burck's observation that *Fragraea littoralis* Bl., with ants near the base, is far less robbed than its comparable sister species *F. oxyphylla* (without nectaries) is valuable. Nieuwenhuis attaches little importance to this observation as she found all flowers of *F. littoralis* robbed. But one would like to know whether she made actual observations and whether in that place ants were absent, as in the abnormal case of *Ipomoea* cited above. Her method of just counting holes in fallen flowers is too rough and her arguments are biased.

A crucial experiment might have been to cultivate the best adapted *Thunbergia grandiflora* first without ants and then with them. Then, however, two complicating factors should be reckoned: (a) the protection offered by the very hard nectar room which may be circumvented by obstructing the normal entry, (b) as the bees learn the method of robbing and the danger of ant guards individually, carpenter bees without experience should be used.

There are many other statements in Niewenhuis' (1907) paper which do not seem to be based on correct observation. That *Vespa analis*, while on the wing, bites holes in a flower is probably a faulty observation. She also does not consider the possibility of punctures being made by birds which can be distinguished from

those made by *Xylocopa*. On the other hand Burck is wrong when he considers flowers as unpollinated when they are found punctured. The legitimate visitor may pollinate them whereas other visitors puncture them.

The facts brought out here, in my opinion, are important in showing not only the role of ants in floralbiology, but also the influence of tropical animals on the evolution of the flower. This factor is likely to be neglected by studies from a temperate standpoint and especially when temperate plants show remnants of these structural peculiarities. Grant (1950) has already demonstrated this in connection with the origin of inferior ovaries and was right in attaching importance to the protection against puncturing by birds and gnawing by beetles.

Myrmecophoby

Many structures in plants have been explained as protections against unwelcome ant visitors. They included mechanical devices in flowers and also extrafloral nectaries, which were believed to divert the attention from the flowers ("Ablenkungshypothese"). The latter idea does not sound probable and moreover, for many of the nectaries near flowers a different explanation has been offered in the previous pages.

Perhaps chemical repellants are important to protect the vegetative parts of plants in regions where *Atta* is a danger. I found no indications of this sort in Java.

The necessity of a provision against invasion of the inner parts of the flower is obvious in those *Xylocopa* flowers which attract ants to the base of the flower tube. It is notable that in *Thunbergia grandiflora* the guarding ants do not crawl over the corolla in order to enter the flower. Previously I had wondered how in *Myrmecodia* plants the flowers could be freely visited by pollinators. The fact is that these white flowers, probably Sphingid flowers, never showed a single ant. The flowers set abundant fruit, but it is not necessary to ascribe this to self pollination as has been done by Burck (1890). His observation that the flowers remain closed may have been made during day time.

The reason why ants never approach the flower tubes of *Myrmecodia* may be that chemical substances in the petals, probably odorous volatile substances, are repellant. To test this, equal large strips of petals and of leaves of some plants with *Xylocopa* connections were placed on the pathways of the common ant *Dolichoderus bituberculatus*, the most frequent species in the ant guards in Java. *Bauhinia purpurea* L. and *Thunbergia grandiflora* Roxb. had the same effect in repeated experiments.

At first leaf strips and petal strips frightened off the ants, as any material would do due to the absence of the trusted odour in the ant path. Very soon however, the behaviour of the ants changed. They came towards the leaves, at first hesitantly, but eventually crossed them. The flower strips, however, were never stepped upon, the ants always passing around them in a wide curve.

That really odour is the repellant is made more probable by further experiments with ornithophilous flowers which seem to have no odour. Flower strips of *Tecomaria capensis* Fenzl. and *Pyrostegia venusta* Miers had no special repelling effect.

In *Bauhinia* the protection against invasion of the nectar holding inner parts of the flower does not seem ideal. The narrow bases of the petals do not surround the nectary completely. Choripetaly is a handicap in this respect. This plant has no ant guard.

Flower odours, considered merely as excreta by the physiologist, were thought by early romantic ecologists as means of inducing ecstatic effects in insects. In most instances this idea has been replaced by the conception of their function as signals, linked with food or as a means of obtaining "Blütenstetigkeit". Perhaps the old idea of ecstatic effects still holds good in some cases where an innate preference in insects and mammals guides them to a flower with such a smell. A new possibility of the significance of odours in the life of the flower, as shown above, should also be considered.

Stäger (1931), in extensive experiments with perfumes and volatile oils from flowers, found that traces of them narco-

tized, frightened and killed ants, labiates and umbellifers having the strongest influence.

I believe that it would be useful if someone repeates his and my experiments on a grand scale taking flowers from different biological classes, preferably from a tropical country.

Indeed, myrmecophoby covers a wider field than merely the plants with myrmecophytic tendencies. Perhaps it should be considered as a more general evolutionary force for plant life in the tropics and should be given more attention in theories about the origin of sympetaly.

Are the mechanical devices in flowers sufficient to explain why ants, which detect the smallest quantity of sugar in a tropical household, are relatively rare on the flowers? Why is such an excellent foodstuff as pollen not always carried away by the omnipresent ants?

In my opinion it is wrong to dismiss negative findings as far-fetched. In *Cucurbita* for instance, when I had been handpollinating flowers of *C. moschata* Duch. in Java and had damaged the flower tubes in the process, hoards of ants entered into the wilted female flowers and took away the pollen from the stigma. In young intact male flowers they were apparently hindered in entering and in intact pollinated female flowers the rolling up of the now obviously non-repellant perianth was sufficient to prevent invasion.

Myrmecochory

This category has acquired general recognition in Europe, since the publication of the monumental monograph by Sernander (1906).

It is inadvisable to speak of the role of insects in general in the dispersal of seeds.

Though the sarcotesta of the " seeds " of pteridosperms and their successors might have attracted dispersers in ancient times, there is no evidence yet that the relation with ants (or other insects) is very old and fundamental. The relation between primitiveness of flowers and pollination by beetles is not paralleled by one between primitiveness and some special seed dispersal. Perhaps seeds and fruits, as we know them now, underwent too many transformations. It is also possible that the primitive seeds were too big in the beginning and could not be dispersed by wind or insects.

One point that needs to be investigated is the influence of elaiosomes on ants. The droplets in the cells consist of some oily substance. Often this permeates cell walls. It has been reported in the sporangia of myrmecochoric ferns. It seems improbable that it is an ordinary fat. Stäger (1933) found ants interested in the crushed mass of fatty seeds but since these ants took all kinds of food, flowers, starchy products like bread, and proteins, it is not convincing that it was fat alone which was responsible for the prompt response of the ants towards elaiosomes. I tried an analysis of the substance of a large number of elaiosomes of *Rottboelia exaltata* L. but have yet no results.

In 1932 I gave an account of the dispersal of the above mentioned grass in addition to several others and also of *Curculigo orchioides* Gaertn. from Java and suggested that myrmecochory was important as a vegetation forming factor in a Javanese savannah region. Another vegetation type that is determined by myrmecochory is the or that one of the ant epiphytes, for details concerning which reference is made to the handbooks of seed dispersal (Ridley, 1930; Ullbrich, 1928).

The researches on Javanese ant epiphytes by Docters van Leeuwen (1929) concern orchids (*Acriopsis javanica* Reinw. and *Dendrochilum pallideflavens* Bl.), ferns (*Polypodium sinuosum* Wall.) and *Lecanopteris carnosa* (Reinw.) Bl. Asclepiadaceae (*Dischidia* spec. div., *Hoya* spec. div.) and others like *Aeschynanthus* species. These researches show one point of special interest, which is that some of the ant epiphytes show a combination of myrmecochory and myrmecodomy, for instance the two ferns mentioned above.

The myrmecochoric adaptations often give the impression of a secondary modification of the original wind dispersal. I sought for signs of the myrmecophytic traits in ant epiphytes and found some myrmecotrophy in the Asclepiadaceae. Zimmerman (1932) mentions leaf nec-

taries of *Hoya* ant *Dischidia* showing facultative myrmecochory.

One of the less regular components of the ant gardens of *Iridomyrmex myrmecodiae* in Java, mentioned but not further investigated by Docters van Leeuwen (1929), is *Procris laevigata*. The juicy, white fruit stands of this plant seem to be ornithochorous and ants do not touch them. As soon, however, as the coenocarpiae are damaged, the ants lick the juicy part, tear off pieces from the fruits and carry them to their epiphytic nests. This facultative relationship leads to more intimate connections.

Another case worth mentioning is that of the famous *Myrmecodia*, where myrmecochory occurs in combination with myrmecophylaxis, myrmecodomy and myrmecotrophy. Certainly the juicy fruit is primarily adapted to bird dispersal. Apart from dispersal over great distances, it is possible to locate a nearby colonization by ants. In Ambon I saw them stowing away the seeds in hooks and corners along their pathways, even in the tuber galleries. They seemed to be interested in seeds only and worked them with their mouthparts. I could detect no morphological adaptation in the direction of myrmecochory but Docters van Leeuwen found oildrops in the seed-coat and the surrounding pericarp. He demonstrated that the presence of numerous seedlings near the mother plant was due to the activity of ants.

Besides the plants (especially the ant epiphytes) mentioned above, the handbooks of Ridley and Ullbrich mention a number of tropical myrmecochores. To this the following may also be added:

In *Lochnera rosea* (L.) Eckb., Steenis (1934) noted transport by ants, especially by the species *Cardiocondyle nuda* Meyr. The seeds showed no morphological adaptations, and had neither sugar nor oildrops in the testa. *Cleome ciliata* Schum. & Thonn. was found (Beumee, 1929) to be dispersed in an attempt on the part at ants to obtain elaiosomes containing oildrops. Both the plants mentioned above are weeds in Indonesia. *Datura fastuosa* L. has well-developed elaiosomes which attract ants. The fruit wall breaks at maturity into irregular pieces, thus liberating the seeds. This deviation from the normal type of capsule, as is present in *Datura stramonium*, is in conjunction with myrmecochory.

Clerodendron incisum var. *macrosiphon* Baker has also been described as myrmecochorous.

Nordhagen (1932) analysed the myrmecochory of different *Roscoea* species from mountainous regions in India. He described vividly the typical spontaneous reaction of ants to the elaiosomes formed by the aril. The myrmecochory here was thought to be a secondary transformation from bird dispersal by means of a juicy, red aril.

I described one new case, *Desmodium gyroides* D.C. (1952), which deserves some special attention. The elaiosome is the small, white arillus, usually quite rudimentary in the family. Corner (1953) believes that the family originally had an aril which served as a means of attraction. To the suggestion, to which I agree, that a juicy seed coat may be still more primitive, Corner's objection is that there are plants like *Sterculia macrophylla* with a sarcotesta and a minute aril. He considers it unlikely that a species should be evolving a new feature as a useless rudiment and concludes therefrom that the aril must be a relic and older than the sarcotesta. In my opinion however, a small aril is not useless. In *Desmodium* and probably other plants it is useful for attracting ants. Marloth (*see* Ridley, 1930) even mentions *Sterculia alexandri* spread in this way. The question as to whether bird dispersal or insect dispersal is more primitive does not concern us here but the fact that even in Europe seed coat and arilloid structures are important in insect dispersal may be significant.

The last instance of myrmecochory to be discussed here is in the genus *Turnera*. Corner describes a small rudimentary aril in this plant. Here again it is an aril, large or small which attracts ants. The seeds of *T. ulmifolia* L. var. *elegans* Urban in Ceylon are carried away by ants. The same is established in Java for *T. ulmifolia* C. and *T. subulata* T.E. Sm. (synonymous with the species described by Lock, 1904). There is one special point of interest in these *Turnera* species. Most myrmecochores bring their diaspores into contact

with ants by producing fruits near the ground, curving their fruit stalks towards the ground or dropping their diaspores. The dropping is reflected in a curious deviation from the common type of pod in *Desmodium gyroides* (slits in the side) and is very obvious in *Rottboellia* and allied grasses where the ripe stalks break into pieces (ecological morphology). Now *Turnera* has the seeds in capsules which do not dehisce, but remain upright. Where does it fit in them ?

Perhaps it is not too out of place to relate that both species have very conspicuous leaf nectaries, due to which the ants run over the plant. Lock (1904) says that the ant *Camponotus mitis* Smith visited the leaf nectaries and occasionally carried away the seed. Urban (1883) mentions that only those leaves secrete nectar that are " grown together " with the flower-stalks and that the secretion lasts from shortly before anthesis until 1-2 days after. This makes him think about " Ablenkung ". I saw diverse species of ants visit leaf nectaries and regularly carry away seeds from just opened fruits on the same plant. This exemplifies a co-ordination between myrmecotrophy and myrmecochory recorded on page 193. Sernander (1906) and Nordhagen (1932) have already demonstrated co-ordinations of this kind. The view that myrmecochory is not just a loose connection with an incidental detail in the seed but that it affects the whole organization of the plant seems to have a wider application in the field of myrmecophytism.

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THE EMBRYOLOGY OF *ARGEMONE MEXICANA* L. — A REINVESTIGATION

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Introduction

The earliest contribution to the embryology of *Argemone mexicana* is by Joshi (1933) who has given an account of megaspore formation and development of embryo sac. He has reported the occurrence of T-shaped megaspore tetrads, early degeneration of synergids, large antipodal cells showing signs of degeneration at the time of fertilization and possibility of the formation of endosperm nuclei even before fertilization. Joshi (1932) has also described some abnormal flowers in which the stamens were transformed into leafy structures and the gynoecium had split into 3-5 free leafy carpels.

Bose & Banerji (1933) published a short account of the female gametophyte of the same species. They report that T-shaped megaspore tetrads are of comparatively rare occurrence and that normally a linear tetrad is produced. They also made chromosome counts from the meiotic stages of microspore mother cells and determined the haploid number as fourteen (n=14). Subsequently, Bose (1937) gave a detailed account of meiosis in pollen mother cells. He reported multinucleate tapetum, tetrahedral microspore tetrads and uninucleate pollen grains at the shedding stage.

Recently, Souèges (1949) has given an account of the embryogeny of this plant. He finds that occasionally the segmentation of the suspensor part of the proembryo leads to the formation of an additional proembryo which develops side by side with the normal embryo.

Since a complete account of the life history of this plant was not available, the present investigation was undertaken at the suggestion of Professor P. Maheshwari.

Material and Methods

The material was collected during the years 1951-1952 from different localities of Delhi. Buds, flowers, fruits and seeds of all stages were fixed in formalin-acetic-alcohol and in Nawaschin's fixative. The ovary wall was scraped with a blade to remove the stiff spines. Mature seeds were treated with hydrofluoric acid (10 per cent in 70 per cent alcohol) for a period of two weeks to soften the testa. The customary methods of dehydration and imbedding were followed. Sections were cut 5-20 microns thick and stained

in safranin-fast green as well as Heidenhain's iron-haematoxylin.

For a study of the antipodal cells and early stages of endosperm development, embryo sacs were dissected out from ovules of different ages under a binocular microscope.

Pollen cultures were made on a sugar agar medium (*see* Maheshwari & Wulff, 1937). The pollen tubes were fixed in formalin-acetic-alcohol and stained with acetocarmine and with iron-haematoxylin.

Observations

Flower — The structure of the flower generally resembles that given in the usual texts on systematic botany. Duthie (1903) mentions 2-3 sepals, 4-6 petals and 4-6 parietal placentae, whereas my observations show the number of sepals to range from 2 to 4, petals from 3 to 8 and of the placentae from 3 to 6.

A number of transformations of the floral parts were observed:

i) stamens into leafy structures,
ii) stamens into carpellary structures with marginal stigmatic surface and naked ovules, and
iii) carpellary tissue forming anther lobes.

The transformation of stamens into leafy structures by the gradual elimination of the anther lobes is of common occurrence. This may happen in a large number of stamens in a single flower. The connective flattens and the vascular strand becomes branched. The anther lobes next split into smaller segments separated by sterile regions of the connective. In other stamens only a small portion of the anther lobe is left at the junction of the filament with the connective and in still others, the connective may be completely devoid of anther lobes. Often the transformed stamens become large and somewhat twisted, and occasionally bilobed in the apical region (Figs. 1-5).

In some flowers, 1-3 stamens were seen to have transformed into carpellary structures. They remained healthy and persistent even when the normal stamens had withered away. In the initial stages of such transformations, the connective becomes broad and flattened, often developing 2-3 spines on its outer surface, a feature characteristic of the ovary wall (Fig. 6). In advanced cases of transformation the connective increases considerably in size and resembles the ovary wall in its fleshy nature, in the presence of chlorophyllous tissue, and the occurrence of stomata. The increased size of the connective is accompanied by a well developed vascular supply which is formed by the branching of a single vascular strand. The laticiferous ducts, normally absent in the fertile stamens, are present in the transformed ones. The filaments of such stamens also become broad, fleshy and green, but do not show any spines.

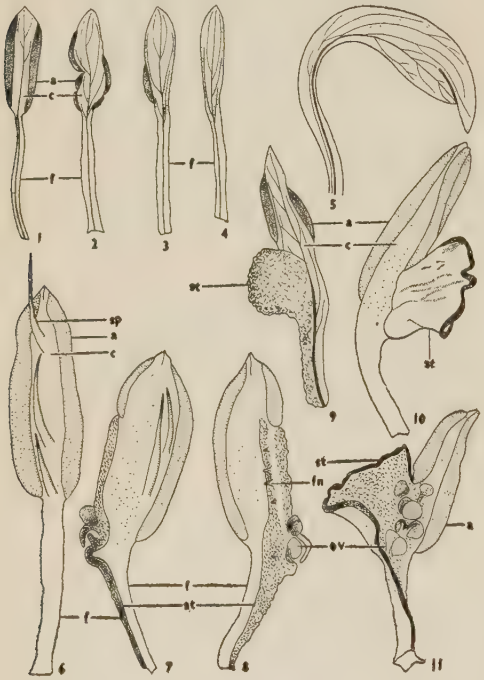
In the transformed stamens there is a gradual dwindling of the anther lobes and in extreme cases only a small part of the anther lobe with just two loculi is left (Figs. 7, 9, 10, 12). The pollen grains in these loculi were devoid of nuclei and seemed to be sterile.

The stigmatic surface arises as a marginal outgrowth in place of the anther lobes. It may be seen either in the form of a prominent flap-like structure on one side extending marginally to the filament, or stigmatic lobes of varying sizes appear along the two margins of the transformed stamen (Figs. 10, 11, 13). In all such cases the stigmatic surface presents a red felt-like appearance and bears papillate hairs on its outer surface, showing considerable resemblance to the normal stigma.

The ovules can be seen on the inner surface of the connective and also in between the stigmatic lobes. The partially transformed stamen bears 2-6 ovules, or none (Figs. 8, 9, 11). In one instance, besides two ovules, there were 4 funicular stalks which failed to develop any ovules (Fig. 8). In those where the staminal transformation into a carpellary structure is almost complete, as many as 17 naked ovules are present (Fig. 13). These are anatropous, broad at the base, white in colour and those which were cut in the median plane showed organized 8-nucleate embryo sacs (Fig. 14).

In some flowers there was a transformation of the carpellary tissue into microsporangia. Here a proliferation had occurred on the outer surface of the ovary wall. This accessory structure of gynoe-

cium had a swollen placenta bearing normal ovules and anther lobes containing sterile pollen grains along the margin (Fig. 15).



FIGS. 1-11 — (*a*, anther lobe; *c*, connective; *f*, filament; *fn*, funiculus; *ov*, ovule; *sp*, spine; *st*, stigmatic surface). Figs. 1-5. Transformation of stamens into leafy structures. Fig. 6. Stamen with three spines on the outer surface of connective. Fig. 7. Stamen with broad connective bearing two spines; more than half the anther lobe has been replaced by the stigmatic surface which extends up to the base of the filament. Fig. 8. Inner surface of stamen, represented in Fig. 7. Note the two anatropous ovules and four funicular stalks in the transformed region. Fig. 9. Stamen in which only a portion of the two anther lobes is left. The broad connective shows branched vascular supply and stigmatic flap on one side. Fig. 10. Back surface of stamen to show part of anther lobe replaced by a large stigmatic flap. Fig. 11. Inner surface of stamen, shown in Fig. 10, with six anatropous ovules in the region of the connective. All Figs. × 6.

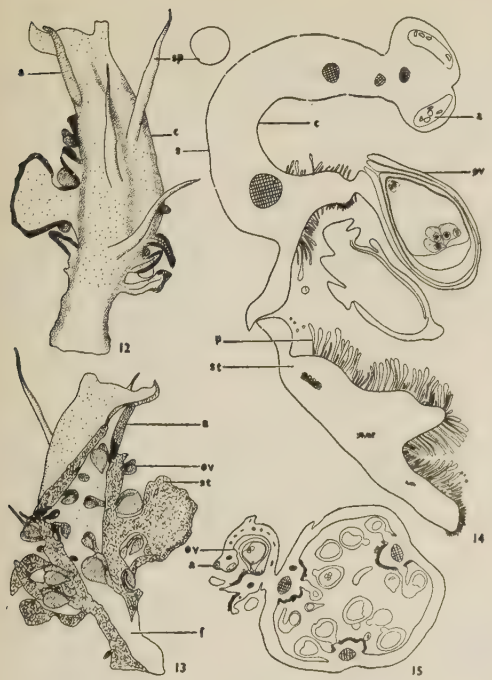
MICROSPORANGIUM AND MALE GAMETOPHYTE — A typical anther shows four microsporangia (Fig. 16), but sometimes five to six microsporangia were also observed. Such a condition may be derived by the splitting of one or two microsporangia at an early stage of development (Figs. 17, 18), or by the fusion of adjacent anthers (Fig. 19).

The anther wall consists of 5-6 layers: epidermis, endothecium, 2-3 middle layers and the tapetum. The epidermis persists up to the time of dehiscence. The endothecium develops the usual fibrous thickenings. Of the middle layers the inner 1-2 are crushed during tetrad formation, but the outermost persists and some of its cells develop fibrous thickenings similar to those of the endothecium (Fig. 24). A few cells in the region of the connective may also show fibrous thickenings.

The tapetum is uninucleate to begin with, but becomes multinucleate owing to mitotic divisions (*see* also Bose, 1937). The nuclei may fuse and divide again resulting in two or more large, amoeboid polyploid masses (Figs. 21-23). Nuclear divisions are sometimes followed by wall formation, so that the tapetum becomes two or more layered at places (Fig. 20). During meiosis in pollen mother cells the tapetal cells enlarge considerably and protrude into the pollen sac. Granular markings appear on the inner surface of the tapetum as well as the middle layers. At the two-celled stage of the pollen grains, the tapetal cells lose contact with one another and their cytoplasm becomes greatly vacuolated. They are consumed and obliterated in the mature anther.

The dehiscence of the anther occurs along the line of partition between the two microsporangia on each side of the anther, where the cells remain narrow and thin-walled. Later, the two microsporangia become confluent due to the disappearance of the transverse septum, while the two segments of the anther wall diverge so as to liberate the pollen grains.

Each anther lobe has 2-3 layers of compactly arranged microspore mother cells as seen in a transverse section. During the initiation of meiosis I, the microspore mother cells increase in size and become vacuolated. No wall is laid between the daughter nuclei, but the cytoplasm becomes densely stained in the middle part of the dyad cell (Figs. 25-28). It persists even during meiosis II and only disappears, when the four daughter nuclei of the tetrad have been orga-



FIGS. 12-15 — (*a*, anther lobe; *c*, connective; *f*, filament; *ov*, ovule; *p*, papillate hair; *s*, stomata; *sp*, spine; *st*, stigmatic surface). Fig. 12. Advanced stage in transformation of stamen, the connective has changed into carpellary structure with three spines on the outer surface. Only a small part of one anther lobe is recognizable. × 5. Fig. 13. Inner surface of stamen, shown in Fig. 12, with stigmatic lobes of varying sizes. Ovules are scattered on the inner surface of the connective and some are also present in between the stigmatic lobes. × 5. Fig. 14. T.s. transformed stamen with two microsporangia on one side and a papillate stigmatic flap on the other, note two ovules in the region of the connective, one with an 8-nucleate embryo sac. × 35. Fig. 15. T.s. ovary with proliferated wall bearing two microsporangia on one side and an ovule on a swollen placenta at the other. × 8.

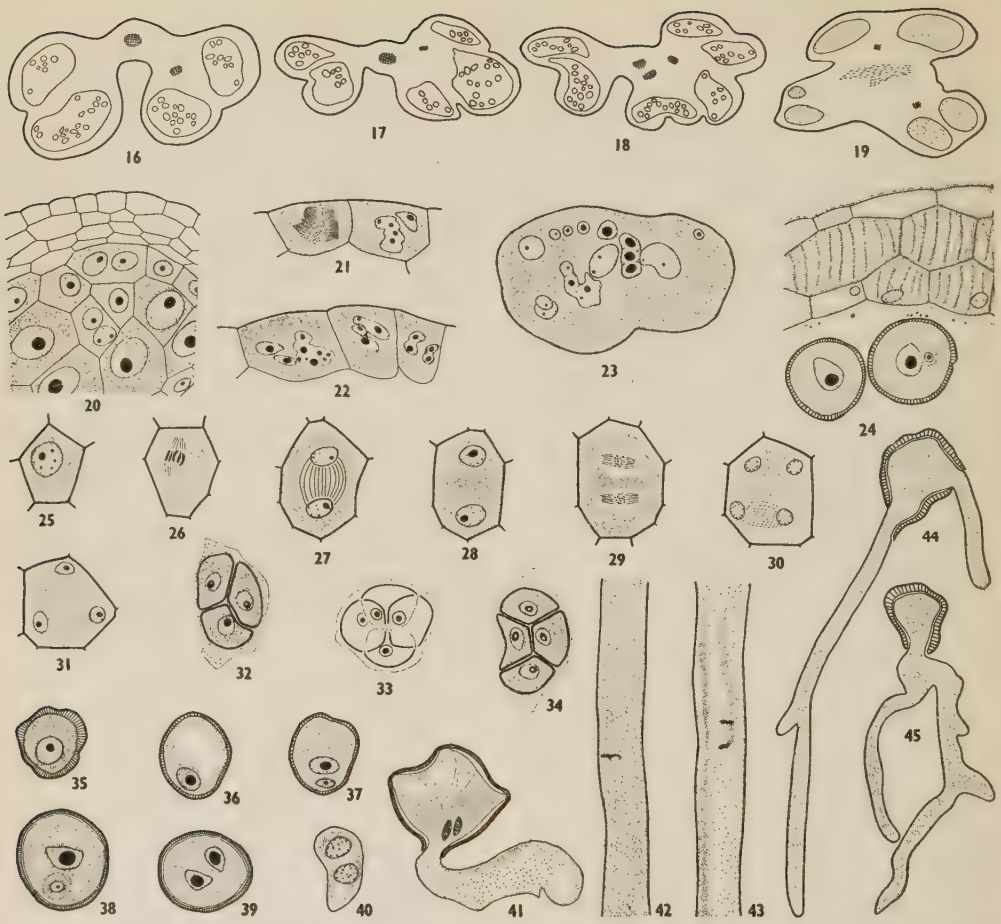
nized (Figs. 29-31). Wall formation occurs by furrowing resulting in the formation of either tetrahedral or decussate type of tetrads (Figs. 32-34), although Bose (1937) mentions only the former type.

Soon after tetrad formation the mucilaginous substance is consumed and the microspores separate (Fig. 35). As they increase in size, the nuclei move to the wallward side where the first division occurs to cut off the generative cell (Figs. 36, 37, 38). The pollen grains differ in size. A large number remain small, their nuclei degenerate and they stain very lightly. They are evidently sterile. Some double pollen grains were observed in which the microspore nucleus had divided to produce two large nuclei of similar size (Fig. 39). One pollen grain which was devoid of the thick exine showed two nuclei separated by a vacuole and looked somewhat like a 2-nucleate pollen embryo sac (Fig. 40).

Mature pollen grains were germinated in cavity slides by the hanging drop technique. Different concentrations of sugar agar media were tried. A mixture of 8 per cent sugar and 1 per cent agar gave satisfactory results and showed 58-60 per cent germination. The generative nucleus usually divides in the pollen tube (Figs. 42, 43), but occasionally also inside the pollen grain (Fig. 41). The tube nucleus could not be observed in the pollen tubes and is likely to have degenerated at an early stage.

MEGASPORANGIUM AND FEMALE GAMETOPHYTE — The ovules are crassinucellate and bitegmic. They arise as small protuberances and become anatropous by the time the megaspore tetrad has been formed (Figs. 46-50). A parietal tissue is produced by the divisions of the primary parietal cell, but a few apically situated cells of the nucellar epidermis may also undergo a solitary periclinal division. At the mature embryo sac stage the micropylar part of the nucellus forms a prominent conical beak (Fig. 50) which is consumed during post fertilization development of the ovule.

The micropyle is narrow and is formed by both the integuments which are swollen at their tips. The exostome may or may not be in line with the endostome, so that at times the micropylar canal has a somewhat zigzag outline (Fig. 50). Occasionally the inner integument fails to reach the apex of the nucellus with the result that the micropyle is formed by the outer integument only. Sometimes the inner integument remains short on one side and the nucellus comes to lie in direct contact with the outer integument (Fig. 51).

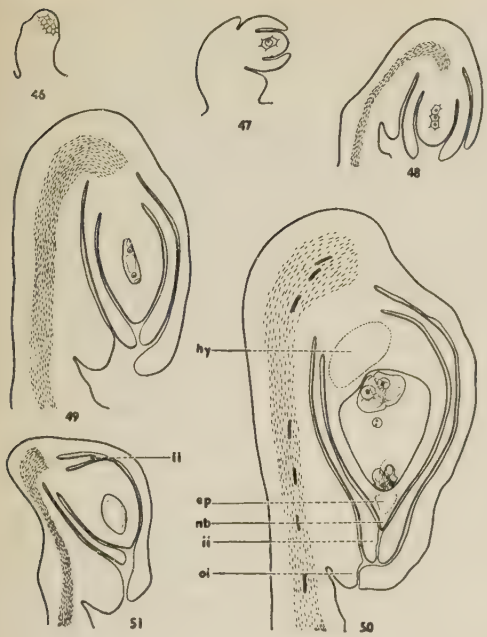


FIGS. 16-45 — Fig. 16 T.s. normal anther with four microsporangia. × 53. Figs. 17, 18. Stamens with five and six microsporangia respectively. × 53. Fig. 19. Abnormal anther, for explanation, *see* text. × 53. Fig. 20. Anther wall at microspore mother cell stage. × 500. Figs. 21-23. Tapetal cells with fusing nuclei. Fig. 21 shows division of a polyploid nucleus. × 500. Fig. 24. Anther wall at mature pollen grain stage. × 500. Figs. 25-34. Stages in the formation of microspore tetrads. × 500. Figs. 35, 36. Uninucleate microspores. × 500. Figs. 37, 38. Bicelled pollen grains. × 500. Fig. 39. Double pollen grain with two similar nuclei. × 500. Fig. 40. 2-nucleate pollen embryo sac. × 500. Fig. 41. Germinated pollen grain (from the stigmatic surface) with two male cells in the body of the pollen. × 500. Figs. 42, 43. Portions of pollen tubes from artificial cultures showing division of generative nucleus. × 500. Fig. 44. Pollen grain showing two pollen tubes. × 313. Fig. 45. Germinated pollen grain with branched pollen tube. × 313.

An interesting feature of the ovule is the presence of stomata[1] in the region of the funiculus (Figs. 117, 121). Stomata are also known to occur on the outer integument of *Cleome, Isomeris* (Orr, 1921), *Nerine curvifolia* (Schlimbach, 1924), *Hymenocallis occidentalis* (Flint & Moreland, 1943) and *Gossypium* (Seshadri Ayyangar, 1948), but their exact role is not understood.

The vascular supply in the funiculus is discernable at the megaspore tetrad stage and extends only up to the chalaza

1. Joshi (1932) recorded the presence of stomata in some abnormal ovules, but he seems to have missed them in the normal ones.



FIGS. 46-51 — (*ep*, epistase; *hy*, hypostase; *ii*, inner integument; *nb*, nucellar beak; *oi*, outer integument). Figs. 46-50. Orientation of ovule at archesporium, megaspore mother cell, tetrad, 2-nucleate embryo sac and mature embryo sac stages. × 119. Fig. 51. Ovule with arrested growth of inner integument on one side. × 60.

(Fig. 48). The differentiation into xylem and phloem is completed at the mature embryo sac stage.

A group of nucellar cells persists below the nucellar beak and another in the region of the chalaza. They represent the epistase and the hypostase respectively[2] (Figs. 50, 76, 117). The walls of these cells become thickened and the cells are filled with deeply staining granular contents. Both epistase and hypostase can be seen even in mature seeds.

The archesporium originates in the hypodermal layer. Usually a single archesporial cell is differentiated, but frequently there are 2-3. They may lie side by side or one below the other. In some ovules the archesporium was found to comprise a group of a dozen or more cells (Fig. 52). Normally a single cell functions, but sometimes two cells may develop into megaspore mother cells (Figs. 55, 56).

The archesporial cell divides by a periclinal wall forming a small primary parietal cell and a large megaspore mother cell. The former may divide either by a periclinal or by an anticlinal wall (Figs. 53, 54). The daughter cells undergo further divisions, so that the megaspore mother cell is separated from the nucellar epidermis by 3-4 layers, the former now gives rise to the usual dyad and tetrad stages (Figs. 57, 58). Often the upper dyad cell may divide by an oblique or vertical wall forming a T-shaped tetrad (Fig. 59).

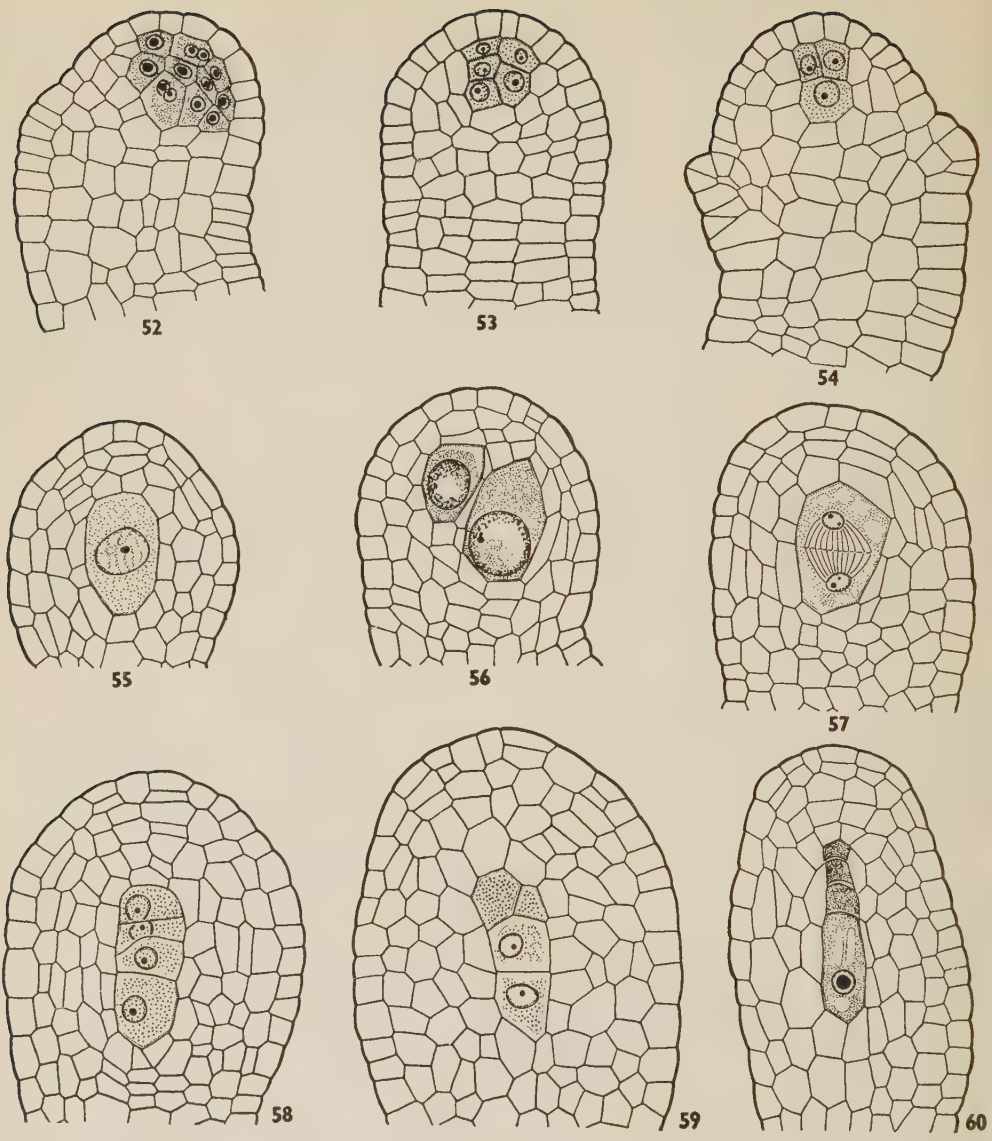
The chalazal megaspore enlarges, becomes vacuolated and its nucleus undergoes three successive divisions to give rise to an 8-nucleate embryo sac which conforms to the Polygonum type (Figs. 60, 61-65).

The synergids are usually hooked and show a filiform apparatus. They are ephemeral and degenerate soon after fertilization, or sometimes even earlier. However, in a number of ovules, one of the synergids was seen to persist (Figs. 68, 74). The egg cell is usually suspended between the two synergids, but occasionally it may be laterally situated. The nucleus lies in its upper part, while the lower is occupied by a vacuole (Figs. 64, 65). The egg thus simulates the synergids in having a basal vacuole. This is the reverse of the condition met with in most angiosperms.

The polar nuclei are similar and fuse before the entry of the pollen tube. It is always the upper polar nucleus which travels down and fuses with the lower polar, so that the secondary nucleus is invariably seen in the chalazal part of the embryo sac close to the antipodal cells (Figs. 64, 65).

The behaviour of the antipodal cells deserves special mention. They are much larger than the cells of the egg apparatus (Figs. 65, 69-71, 73). In each antipodal cell there are one or more large vacuoles towards the periphery, while the nucleus is situated on the inner side where the cells come in contact with one another. During post fertilization stages they continue to increase in size and become

2. Mohan Ram (unpublished) has also found an epistase and hypostase in *Eschscholtzia californica*.



FIGS. 52-60 — Fig. 52. Young nucellus with a group of archesporial cells. × 636. Fig. 53. Formation of parietal cells. × 636. Fig. 54. Megaspore mother cell and primary parietal cell, the latter has divided anticlinally. × 636. Fig. 55. Megaspore mother cell. × 636. Fig. 56. Two megaspore mother cells. × 636. Fig. 57. Dyad. × 636. Figs. 58, 59. Linear and T-shaped tetrads. × 636. Fig. 60. Functioning megaspore. × 636.

greatly hypertrophied (Figs. 74, 77). They show some indications of a haustorial function. Commonly, about 8-10 layers of richly protoplasmic cells of the nucellus lying immediately beneath the antipodals get depleted of their contents and present a collapsed appearance. This is evidently due to the agressive nature of the antipodal cells. It is only at the heart-shaped stage of embryo, or even a little earlier

that they give an unhealthy appearance and their cell walls become somewhat wavy in outline. The scanty cytoplasm present in them gives a light staining reaction and the nuclei are also in the process of degeneration (Fig. 78). Finally, they get completely absorbed by the encroaching endosperm.

The following table gives the size of the antipodal cells at different stages of endosperm and embryo development as studied from whole mounts.

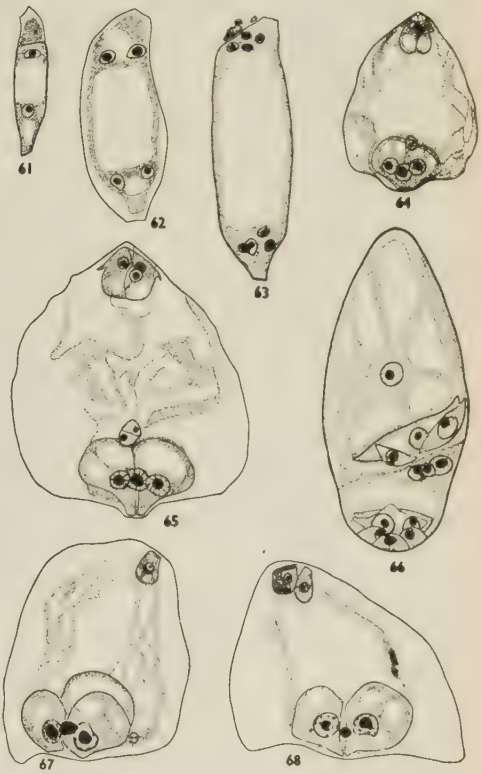
Stage of Development		Length, Breadth of the Three Antipodals (μ)
Mature embryo sac stage	a.	78, 124
	b.	108, 108
	c.	124, 124
Free nuclear endosperm; zygote at resting stage	a.	108, 124
	b.	108, 124
	c.	124, 147
Endosperm cellular; embryo at early globular stage	a.	171, 217
	b.	186, 204
	c.	186, 201
Endosperm cellular; embryo at late globular stage	a.	170, 232
	b.	201, 217
	c.	232, 157

One ovule showed twin embryo sacs lying one above the other. The upper had 4 nuclei out of which 3 had organized into cells at the chalazal end and the fourth was lying free in the middle. The lower embryo sac had 8 nuclei, which organized into 3 small and 2 large cells at the chalazal end and 3 free nuclei at the micropylar end (Fig. 66).

Pollination and Fertilization — Both self and cross pollination seem to occur in nature. Cross pollination is carried out by various insects and bees. In some cases the sepals and petals remained closed, while the anthers had dehisced and deposited their pollen on the surface of the stigma. The ovaries of such flowers develop into normal fruits like those in which cross pollination has taken place.

Observations on germinating pollen grains on the stigma show that although the monosiphonous condition is usual, one may occasionally come across polysiphony. Fig. 44 shows two pollen tubes growing out of a single pollen grain. Rarely pollen tubes may also show branching (Fig. 45).

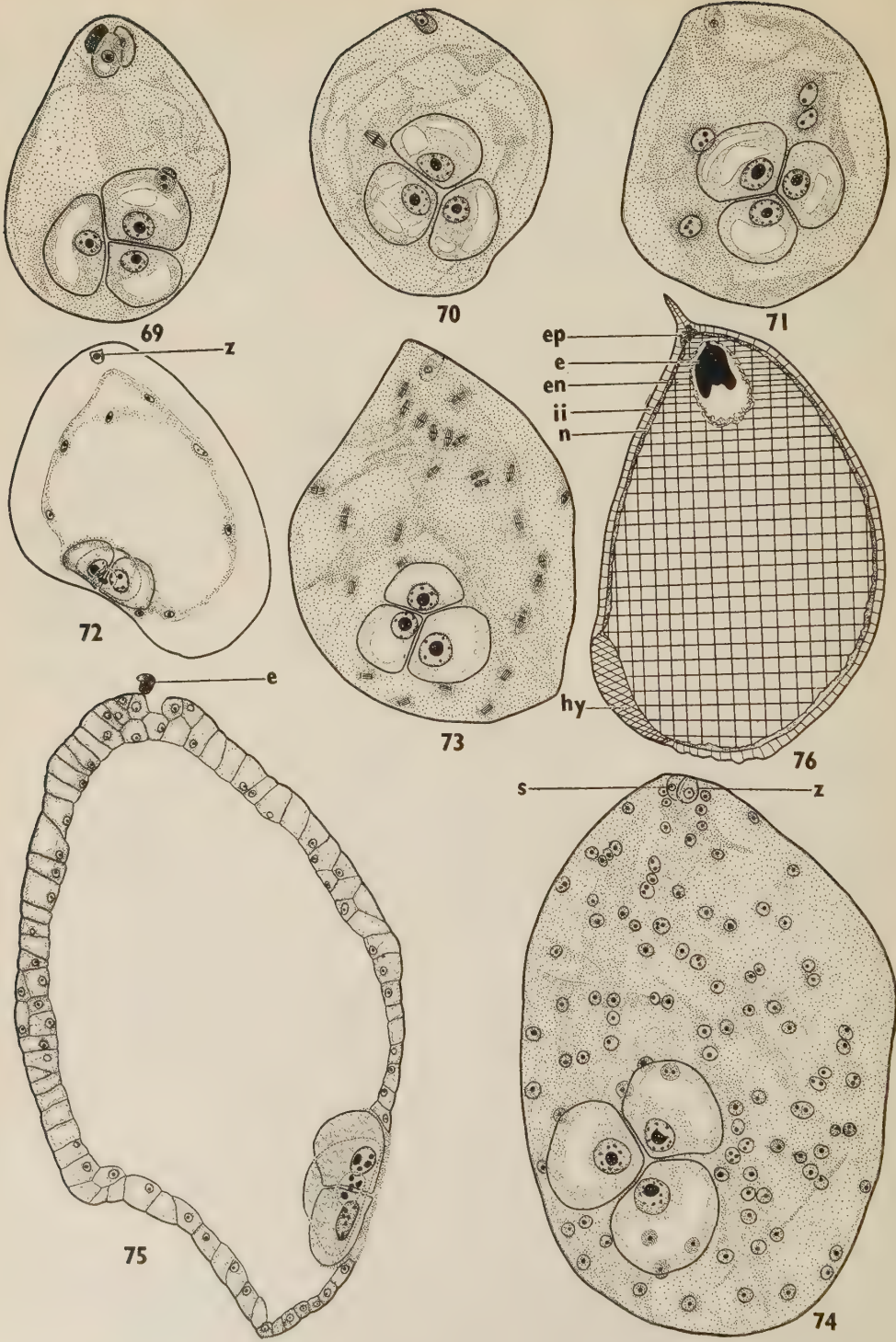
The pollen tubes travel through the short hollow stylar canal which is lined by papillate hairs. These hairs are richly protoplasmic and serve a nutritive role in the growth of the pollen tubes.



Figs. 61-68 — Figs. 61-63. 2-, 4- and 8-nucleate embryo sacs. × 368. Figs. 64, 65. Mature embryo sacs. × 368. Fig. 66. Twin embryo sacs with abnormal organization, details in text. × 368. Fig. 67. Double fertilization. × 368. Fig. 68. Fertilized embryo sac showing undivided oospore, persistent healthy synergid and two endosperm nuclei at metaphase and telophase. × 368.

The entry of the pollen tube is always through the micropylar canal. In one ovule two pollen tubes had penetrated the micropyle, one of which had reached the embryo sac, while the other had taken an abnormal course between the inner and the outer integuments.

Double fertilization was observed. In one preparation one of the male gametes



FIGS. 69-76.

had fused with the egg and the two nucleoli could be seen quite clearly; the triple fusion nucleus was situated at the chalazal end of the embryo sac (Fig. 67; pollen tube and synergids not drawn).

The remnants of the pollen tube may continue to persist in the micropylar canal till after a proembryo of 5-6 tiers has been formed.

ENDOSPERM — After fertilization the embryo sac increases considerably in length as well as in breadth. The primary endosperm nucleus divides much earlier than the zygote. The endosperm is of the Nuclear type (Fgs. 70, 71). In one embryo sac the 2 nuclei formed by the primary endosperm nucleus were seen at the metaphase and telophase stages (Fig. 68). Further divisions of the endosperm nuclei are also non-synchronous (Fig. 73). At the time of division of the zygote there are approximately 80-90 endosperm nuclei. The nuclei take up a peripheral position (Fig. 72) where they continue to multiply in number. Wall formation occurs along the periphery of the embryo sac which forms one and at places 2 layers of endosperm cells. Further divisions in these cells fill the entire sac with cellular endosperm (Figs 75, 76). Finally, the divisions are confined only to the peripheral one or two layers. The cells of the endosperm are uninucleate, highly vacuolate and thin-walled (Fig. 79). They are filled with oil globules at the early globular stage of the embryo (Fig. 80). The cells around the embryo are always in a state of collapse resulting in a little cavity (Fig. 76). At maturity the walls of the endosperm cells may become slightly thickened.

EMBRYO — The zygote enlarges considerably and undergoes a transverse division giving rise to a terminal cell *ca* and a basal cell *cb* (Figs. 81-83). Rarely, due to changed orientation of the spindle it may divide by an oblique transverse wall (Figs. 84, 85). The sequence of divisions after the two-celled proembryo is quite variable:

i) Both *ca* and *cb* divide transversely and produce a uniseriate filamentous proembryo (Fig. 87).
ii) The cell *ca* divides by a vertical wall and *cb* by an oblique transverse wall (Fig. 88).
iii) The cell *ca* divides by an obliquely transverse wall and *cb* by an oblique vertical wall (Fig. 86).
iv) Both *ca* and *cb* divide by a vertical wall (Fig. 89).

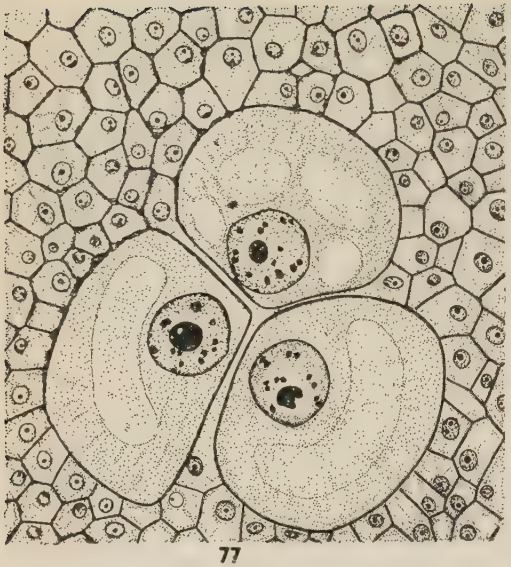
Further divisions are so irregular that it was not possible to trace any definite sequence, but the general course of development is indicated by Figs. 90-102. In a mature embryo the two cotyledons are of similar size, but in some cases, one is slightly shorter. During the elongation of the cotyledons, the embryo consumes the cells of the endosperm lying adjacent to it. The mature embryo is fairly small as compared to the size of the endosperm tissue.

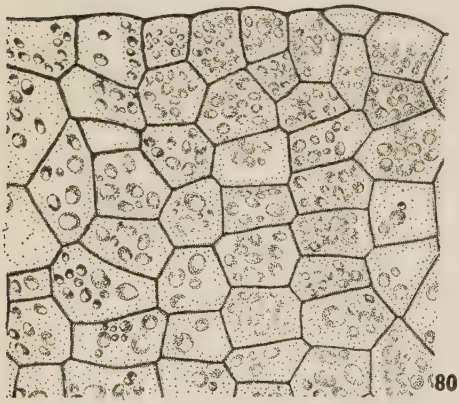
POLYEMBRYONY — As already reported before (Sachar, 1953) in some ovules twin embryos were observed, one being zygotic in origin and the other developing from a synergid.

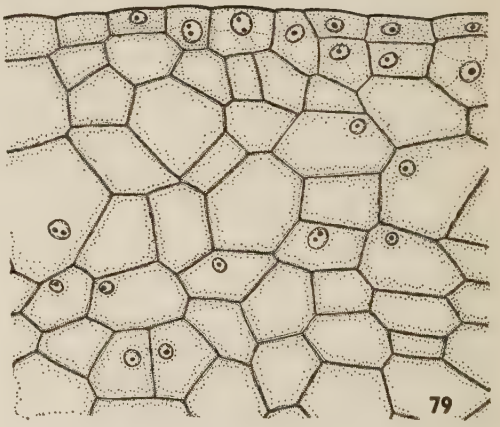
Figs. 68, 74, 103 show one of the synergids persisting by the side of the zygote. The persistent synergid increases in size, its basal vacuole disappears and the nucleus migrates downward. The synergid and the zygote divide simultaneously and form twin two-celled proembryos (Fig. 104). However, the zygotic embryo always grows faster, so that it can easily be distinguished from the synergid embryo by its larger size. The twin embryos may lie in close juxtaposition or they may overlap each other (Figs. 105-107).

←

FIGS. 69-76 — (*e*, embryo; *en*, endosperm; *ep*, epistase; *hy*, hypostase; *ii*, inner integument; *n*, nucellus; *s*, synergid; *z*, zygote). Fig. 69. Mature embryo sac. × 161. Fig. 70. First division of primary endosperm nucleus. × 161. Fig. 71. Embryo sac with four endosperm nuclei. × 161. Fig. 72. Endosperm nuclei arranged along the periphery of the embryo sac. × 161. Fig. 73. Endosperm nuclei in active state of division. × 161. Fig. 74. Free nuclear endosperm, note the persistent synergid. × 161. Fig. 75. Embryo sac with peripheral layer of cellular endosperm. × 161. Fig. 76. Advanced stage of cellular endosperm filling the entire embryo sac cavity (diagrammatic). × 47.



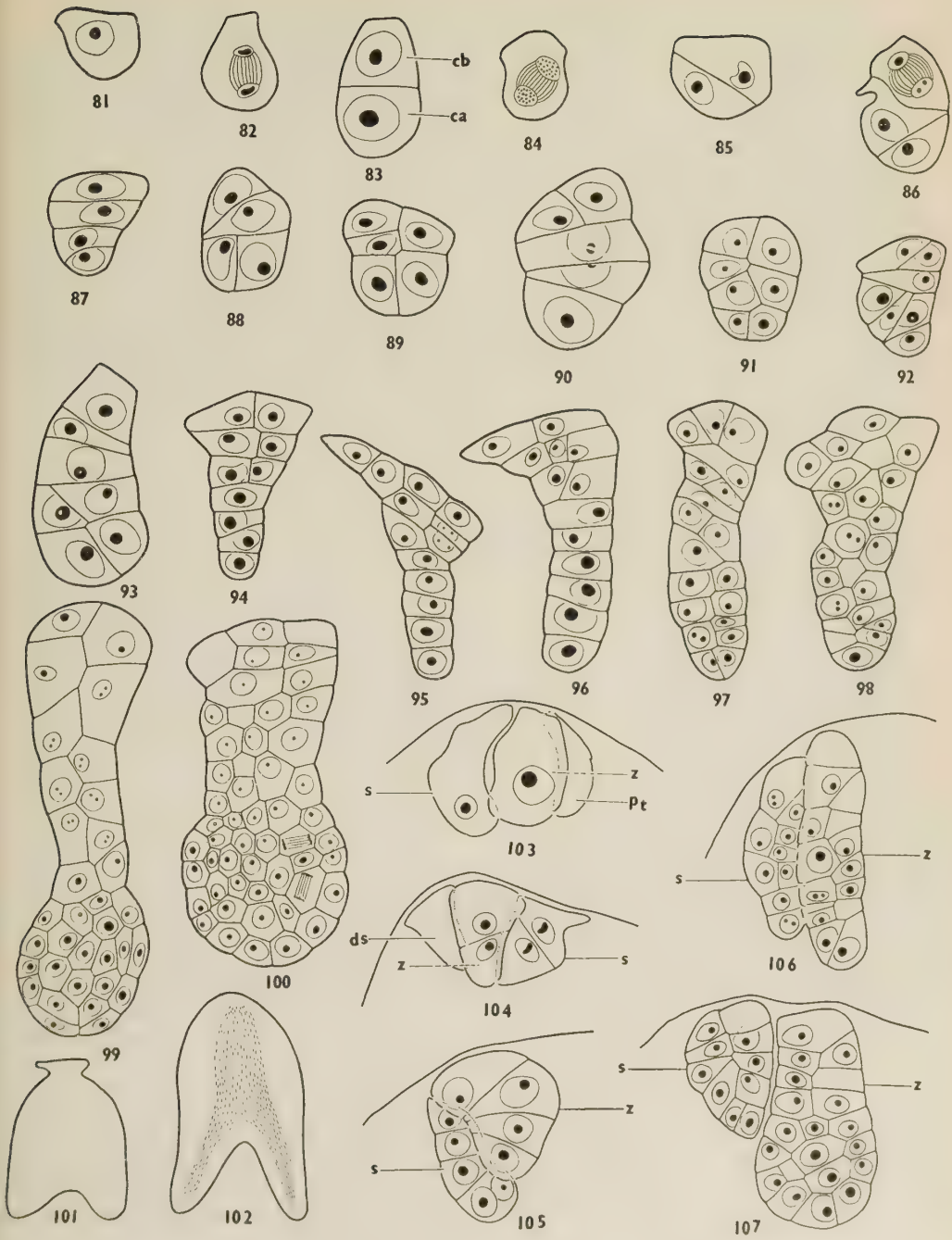




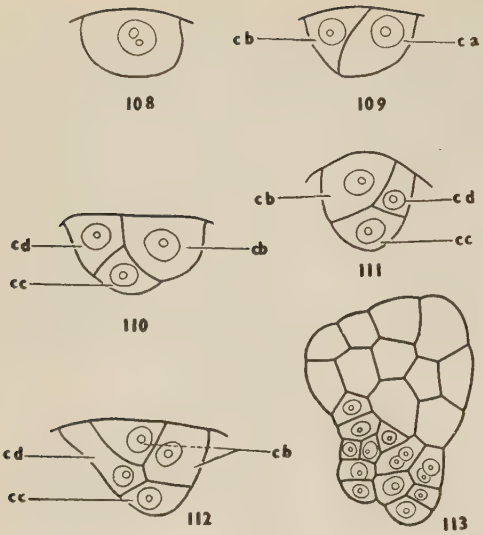
FIGS. 77-80 — Fig. 77. Greatly hypertrophied antipodal cells at the cellular stage of the endosperm. Fig. 78. Antipodal cells beginning to degenerate. Figs. 79, 80. Parts of cellular endosperm enlarged from young and mature seeds, the latter shows food reserves in the form of oil globules. All Figs. × 179.

There was no indication that the synergid is ever fertilized. Only one pollen tube was seen in embryo sacs with twin embryos and in all probability the synergid proembryo is haploid. But it was not possible to confirm this by chromosome counts as suitable mitotic figures were not available.

In several other plants like *Plantago lanceolata* (Souèges, 1926), some species of *Lilium* (Cooper, 1943), *Orchis maculata*, *Platanthera chlorantha*, *Lister ovata* (Hagerup, 1944, 1947), *Arabis lyalli* (Lebègue, 1948) and *Erythraea centaurium* (Crété, 1949), synergids are known to develop into haploid embryos. The synergid embryos of *Argemone mexicana*, in common with those of above mentioned plants, usually degenerate at an early stage of development, but are of considerable



FIGS. 81-107 — (*ds*, degenerating synergid; *pt*, pollen tube; *s*, synergid, or synergid proembryo; *z*, zygote or zygotic proembryo). Figs. 81-102. Stages in the development of the embryo. Figs. 81-100. × 500. Figs. 101, 102. × 156. Figs. 103-107. Twin proembryos at different stages of development. × 500.



FIGS. 108-113 — Reproduced from Souèges (1949) to compare the early stages of embryogeny and formation of twin proembryos, explanation in text. × 430.

interest as a possible source of haploids provided they can be stimulated to develop artificially.

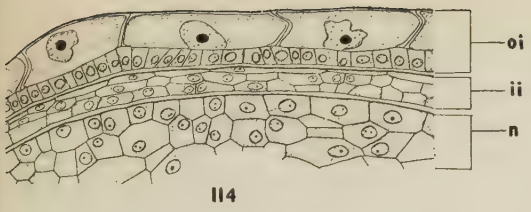
Germinating seeds sometimes gave seedlings with three cotyledons. Such a condition may have been derived either by the splitting of one of the cotyledons or by the fusion of twin embryos at an early stage of development. In one case a seedling showed two pairs of cotyledons each with a distinct plumule, but there was a common radicle. This seemed to be a case of fusion of twin embryos in the lower region.

A polycotyledonous condition is common in gymnosperms, but a few angiosperms like *Impatiens roylei* (Bexon & Wood, 1930), *Morus multicaulis* (Datta, 1941), *Cinnamomum camphora* (Choudhury & Mitra, 1953), *Corchorus capsularis* (Datta, 1954), *Opuntia dillenii* (Maheshwari & Chopra, 1955) and *Ficus religiosa* (Johri & Konar, unpublished) also show the sporadic occurrence of more than two cotyledons. In *Eschscholtzia californica*, Mohan Ram (unpublished) finds the presence of four cotyledons to be a fairly common feature.

SEED COAT — At the mature embryo sac stage the nucellus is 3-4 layers at the sides, but more massive at the chalazal end. The inner integument consists of three layers of narrow cells and the outer of two layers (Fig. 114). Both the integuments are swollen in the region of the micropyle due to the meristematic activity of the cells.

During post fertilization stages, the endosperm enlarges consuming the nucellar cells, so that a single layer is left at the periphery (Figs. 115, 116) which persists even when the embryo has developed small cotyledons. It is only during the further growth of the seed that this layer too gets completely absorbed and is not represented at the ultimate stage. As already mentioned, part of nucellar tissue at the micropylar and chalazal end persists in the form of epistase and hypostase (Fig. 119).

Out of the two integuments the outer is mainly responsible for contributing mechanical strength to the seed coat. The outer two layers of the inner integument degenerate quite early in centripetal order and only their remnants may be observed at the globular stage of the embryo. The cells of the innermost layer of the inner integument which are quite inconspicuous to begin with enlarge in size. Soon they are devoid of their contents, but become cutinized along the inner surface and persist in an empty condition right up to maturity of the seed coat (Figs. 115, 116). In the chalazal end this layer gives an undulating appearance (Fig. 119). Both the layers of the outer integument take part in the formation of the seed coat. The cells of the outer epidermis enlarge, especially along the plane of the ovule. Their nuclei also increase in size and are surrounded by numerous starch grains (Figs. 115, 116) which disappear when the seed is nearly mature. The cells become greatly thickened and develop a strong cuticle along their outer tangential walls. In surface view they show numerous simple pits of irregular outline (Fig. 121). The inner epidermis of the outer integument consists of rectangular cells which are richly protoplasmic (Fig. 115). They enlarge much more along the radial than the tangential direc-



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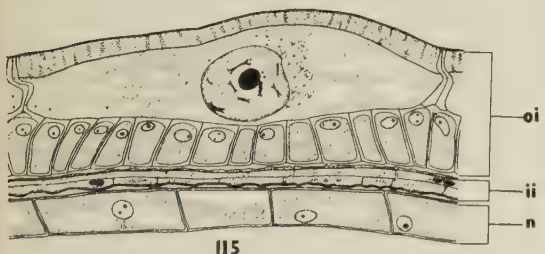

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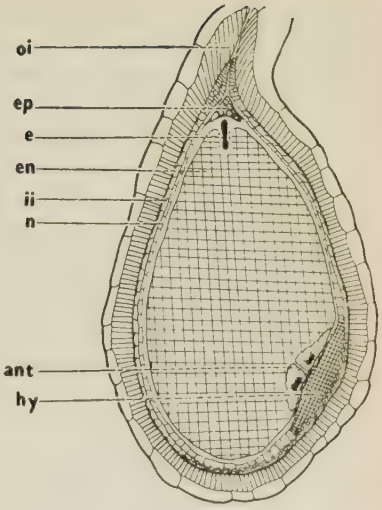

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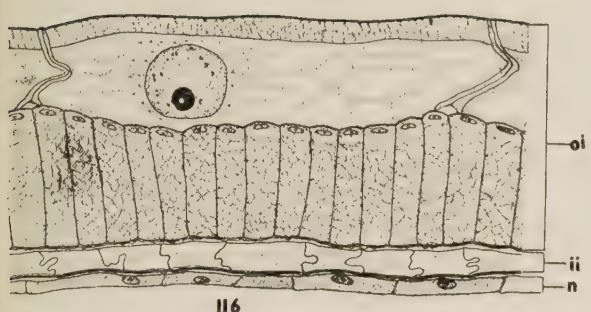

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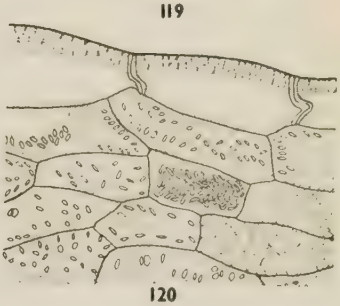

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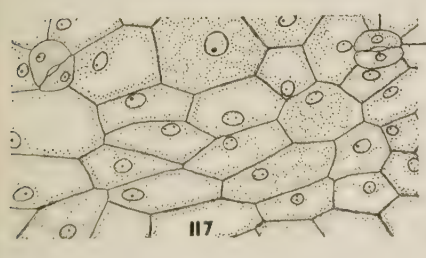

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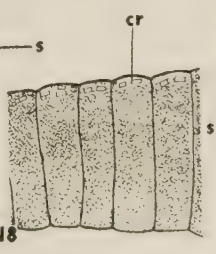

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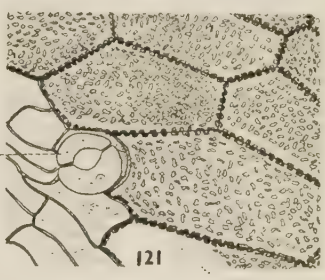

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FIGS. 114-121 — (*ant*, antipodal cells; *cr*, crystalline bodies; *e*, embryo; *en*, endosperm; *ep*, epistase; *hy*, hypostase; *ii*, inner integument; *n*, nucellus; *oi*, outer integument; *s*, stomata). Figs. 114-116, 118. Stages in the development of seed coat. × 370. Fig. 117. Part of funiculus enlarged to show stomata. × 370. Fig. 119. L.s. seed at globular proembryo stage, mark the epistase, laterally placed hypostase and antipodal cells (diagrammatic). × 185. Fig. 120. Same as Fig. 117, at mature seed stage, note the pitted and reticulate thickenings of the cells. × 370. Fig. 121. Portion of funiculus, showing the stomata and the irregular pits on the epidermal cells seen in surface view. × 370.

tion and develop some fibrillar type of thickenings (Fig. 116). The nuclei of this palisade-like layer are situated near the outer tangential walls and disappear at the heart-shaped stage of the embryo. At this time numerous crystalline bodies

also appear in the peripheral zone and the cells get impregnated with tannin-like substance (Fig. 118). In mature seed the cells of the funiculus develop pitted as well as reticulate type of thickenings (Fig. 120).

Discussion

Joshi (1932) described some abnormal flowers of *Argemone mexicana*. They differed from the normal in having persistent sepals, petals and stamens. The stamens showed the following characteristic features: (i) a broad filament and connective, (ii) bristles on the filaments, (iii) stomata and chlorenchymatous tissue in the connective and filament, (iv) absence of a dehiscence mechanism in anther, and (v) occurrence of abortive pollen. In advanced cases of phyllody, the stamens were completely modified into leaves. In my material also, all the transitional stages of stamens into leafy structures were available. In the case of gynoecium, some of the abnormal features described by Joshi are: the presence of a gynophore, a comparatively longer style, freedom of the integuments from the nucellus, long micropyle, development of stomata on the integuments, presence of chloroplasts both in the cells of the integuments and the outer layers of the nucellus, and branching of the vascular trace in the chalazal region. In some flowers the gynoecium opened out, the style and the stigma disappeared and the ovary formed a cup-shaped structure with lobed margins. In advanced cases the lobes were deeper and finally split into free carpellary leaves, devoid of ovules.

Some other examples of teratological interest in which carpellary tissue is transformed into anther loculi or even stamens, have been reported by Chamberlain(1897) and Hagerup (1938) in *Salix*, Rao (1940) in *Dianthus*, Farooq (1952) in *Citrus*, Johri & Tiagi (1952) in *Cuscuta*, and Singh (1954) in *Morus*. Hagerup recorded the following types of transformations in *Salix*:

(i) A part of the carpellary tissue may give rise to a pollen sac. A similar condition is known in *Cuscuta*, *Morus* and *Citrus*. I found the same type of transformation in *Argemone* where the ovary wall bore two microsporangia.

(ii) One or both the carpels may give rise to an anther at the apex with or without any trace of the style and stigma at the distal end.

(iii) In others, one or more ovules may be replaced by an anther.

In *Papaver bracteum* Masters (1868) mentions another type of conversion in which a considerable number of stamens sometimes develop into pistils, especially those which are nearest the centre of the flower. In these flowers the filaments are said to become ovaries, while the anthers are curled so as to resemble the stigmas. Goeppart (1850; quoted in Masters, 1868) noted a similar transformation in *Papaver somniferum*.

My material also showed metamorphosis of stamens into carpellary structures, but unlike *Papaver bracteum* the connective and the anther were transformed into carpellary tissue and the stigmatic surface arose as a marginal outgrowth which extended even up to the base of the flattened filament.

There is a difference of opinion regarding the significance of such teratological specimens. According to Hagerup (1938), the ovule of *Salix* is axial in origin and is a complete and independent organ. He, therefore, considers it to be homologous with the stamen. On the other hand, Johri & Tiagi (1952) hold that such teratological specimens even though of regular occurrence should not be used as a basis for proposing homologies.

Maheshwari (1949) has reviewed the literature on the shedding of uninucleate pollen grains and cites the following examples:

Argemone mexicana (Bose, 1937); *Crotalaria juncea* (Banerji & Samal, 1936); *Corchorus olitorius* (Banerji, 1933); *Carthamus tinctorius* (Banerji, 1940); *Leandra cordifolia* (Subramanyam, 1942); *Pennisetum typhoideum* (Rangaswami, 1935); *Putranjiva roxburghii* (Dutt, 1943); *Santalum album* (Srinivasa Iyengar, 1937); and *Sesamum indicum* (Nohara, 1934). Maheshwari's own studies show that the pollen grains of *Corchorus olitorius*, *C. capsularis*, *C. acutangulus* and *Crotalaria juncea* are shed at the two-celled stage,

and that of *Carthamus tinctorius* at the three-celled stage. The remaining ones, in his opinion deserve reinvestigation.

Narayanaswami (1953) in *Pennisetum typhoideum* reports three-celled pollen grains at the time of liberation. The present study shows that in *Argemone* the pollen grains are, as a rule two-celled at the shedding stage, and only in a few cases an undivided microspore nucleus was observed. Thus it appears that Bose (1937) must have observed immature pollen grains.

Another feature of interest is the occasional presence of double pollen grains having two vegetative nuclei of equal size. In one instance, however, an elongated pollen grain without any exine very much resembled a small 2-nucleate pollen embryo sac.

There is a characteristic nucellar beak, an epistase and a hypostase which were missed by earlier workers (*see* Joshi, 1933; Bose & Banerji, 1933).

Joshi (1933) reported a single archesporial cell which divides by a transverse wall to form a primary parietal cell and the megaspore mother cell. The former divides by an anticlinal wall and the daughter cells undergo 1-2 periclinal divisions. Later, Bose (1937) also reported a single-celled archesporium with two-cells occurring as an unusual feature. He, however, contradicted Joshi with regard to the plane of the first division of the parietal cell and said that it is periclinal and not anticlinal. My study shows that the archesporium is not always single-celled, but even when there is a many-celled archesporium only a single cell is functional. Occasionally, more than one archesporial cell may divide periclinally resulting in two megaspore mother cells. The first division of the primary parietal cell may be periclinal or anticlinal.

Joshi (1933) figured only T-shaped tetrads as a characteristic feature of this plant, while Bose & Banerji (1933) hold that a linear tetrad is of common occurrence. In my material, I have observed both linear as well as T-shaped tetrads.

The embryo sac is of the Polygonum type as reported earlier. According to Bose (1937) the synergids are pear-shaped, and the egg nucleus is situated at the top with a basal vacuole. The synergids examined by me mostly showed hooks and a filiform apparatus. The egg nucleus lies at the top as observed by Bose (1937). Joshi (1933) mentions that the synergids disorganize at an early stage. Bose & Banerji (1933) also reported that the synergids are not seen after fertilization. My observations, however, show that the behaviour of the synergids is rather variable. They may degenerate before or after fertilization, but sometimes one of the synergids may persist during the development of the endosperm. A similar feature has been noted by Mohan Ram (unpublished) in *Papaver rhoeas*. Huss (1906) found that sometimes both the synergids persist in *Hypecoum procumbens*, even when a proembryo of seven tiers has been produced.

The description of the antipodal cells given by previous workers deserves closer examination. Joshi (1933) reported that at the time of fertilization, the antipodals are 200 microns in diameter. At this stage disorganization had already set in, as could be seen from the disappearance of their nuclear membranes. The protoplasm is of alveolar nature and the nucleolus had broken up into a number of darkly staining masses indicative of disintegration. According to Bose & Banerji (1933) the signs of degeneration of antipodal cells are just apparent, when the free nuclear endosperm forms a lining layer next to the wall of the embryo sac. I studied the behaviour of the antipodal cells with the help of microtome sections as well as dissections, and have observed that they continue to enlarge and attain a maximum size, when the endosperm has become cellular and the embryo is at the globular stage. The antipodals become vesicular and do not show any breaking down of their nuclear membranes or their nucleoli even at the cellular stage of the endosperm. At mature embryo sac stage the nuclei of the antipodal cells have a nucleolus and several small peripherally disposed chromatic bodies. With the increase in size of the nucleus, they also become fairly prominent. I am unable to agree with Joshi's statement that the chromatic bodies arise by the disintegration of the nucleolus.

Large persistent antipodals are also known to occur in a few other plants like *Aconitum napellus* (Osterwalder, 1898), *Caltha palustris* (Grafl, 1941), *Fumaria, Corydalis, Papaver* (Huss, 1906) and *Fumaria parviflora* (Saksena, 1954). In *Caltha* they attain a high degree of polyploidy by the division of the nucleus and the subsequent fusion of the daughter nuclei. The considerable increase of the antipodals in the present case suggests their high metabolic activity. They draw nourishment from the adjacent nucellar cells which become depleted of their contents.

Joshi (1933) reports a precocious development of the endosperm. He states, " The formation of endosperm had set in all the ovules of the older ovaries examined by the writer whether there was a trace of the penetration of the pollen tubes or not and the fertilization had taken place or not. The egg has not yet divided when a good deal of the endosperm has been formed. This leads one to suspect that here also the formation of endosperm may be beginning even before fertilization as reported by Coulter (1898) for *Ranunculus*." My preparations show quite clearly the remnants of the pollen tube in the micropylar canal of all the medianly cut ovules containing early stages in endosperm formation. Further, no endosperm developed in ovules of flowers which had been castrated and bagged.

According to Souèges (1949) the zygote of *Argemone mexicana* divides by an oblique vertical wall forming the basal cell *cb* and the terminal cell *ca* (Figs. 108, 109). The terminal cell *ca* divides obliquely to give rise to *cc* and *cd*. Thus a three-celled proembryo is formed in which the lower cells *cb* and *cd* support the cell *cc* (Figs. 110, 111). In its origin and arrangement it resembles very much that of *Hypecoum procumbens* (Souèges, 1943). The cell *cb* further divides by an oblique vertical wall (Fig. 112).

My study of embryo development in *Argemone* shows that there is no strict sequence of divisions as pointed out by Souèges (1949). Usually, the zygote divides by a transverse wall and only in a few cases I found it to be obliquely oriented. Further, the sequence of divisions in two-celled proembryos is extremely variable, so that it is not possible to place it in any definite type. Both *ca* and *cb* may divide transversely, so as to produce a linear four-celled proembryo, or both may divide by a vertical wall forming a quadrant-like structure. In still other cases, *ca* divided by an obliquely transverse and *cb* by an obliquely vertical wall, or *ca* divided by a vertical wall, and *cb* by an obliquely transverse wall. Since such variations are fairly common in my material I am unable to confirm the course of events described by Souèges.

Souèges (1949) has also stated that sometimes a second rudimentary embryo is formed due to the segmentation of the massive suspensor[3] (Fig. 113). Twin embryos have also been observed by me, but while the larger embryo is derived from the zygote, the smaller originates from a persistent synergid. The evidence in favour of this view may be summarized as follows:

(i) A number of post fertilized embryo sacs having numerous free endosperm nuclei showed a healthy and enlarged synergid by the side of the zygote.

(ii) Twin embryos have been traced back to such early stages where even the basal cell of the zygotic proembryo had not undergone any division, showing that the second proembryo is initiated long before the formation of the suspensor.

(iii) Souèges has shown a common suspensor for the twin proembryos. Such an interpretation could also be due to intimate juxtaposition of two distinct embryos which give a false impression of cleavage embryony (*see* also Fagerlind, 1944). In my preparations, I found two distinct embryos which either lay side by side or overlapped each other. It is concluded that Souèges has misinterpreted the synergid embryo as a cleavage product of the zygotic embryo.

Summary

The flowers are tri- or tetra-merous but sometimes the number of petals may vary

3. To quote Souèges " chez l'*Argemone*, ils se segment et produisent un suspenseur masif, ...; quelquefois enfin leurs segmentations conduisent à la formation de veritables proembryons rudiemntaires se développant à côté de l'embryon normal (fig. 18, 19)."

from three to eight. Occasionally, the stamens become transformed into leafy or carpellary structures. In the latter naked ovules are borne on the expanded connective, while its margin shows stigmatic papillae. Sometimes the ovary wall proliferates and develops a lateral anther lobe.

The wall of the anther comprises the epidermis, fibrous endothecium, 2-3 middle layers and glandular multi-nucleate tapetum. The latter may become two- to three-layered at places. The outermost middle layer persists and some of its cells as well as those of the connective develop fibrous thickenings. Reduction divisions are simultaneous and tetrads may be of the tetrahedral or decussate type. The pollen grains are shed at the two-celled stage. As a rule they are monosiphonous, but sometimes a polysiphonous condition and branching of the pollen tubes may also occur.

The ovules are anatropous, bitegmic and crassinucellate and the nucellus forms a conical beak. The funicular epidermis shows stomata. The hypodermal archesporium may be one to many-celled, but usually only one cell functions. Its first division cuts off a parietal cell which multiplies to produce the parietal tissue. The megaspore mother cell gives rise to a linear or T-shaped tetrad and the chalazal megaspore produces an 8-nucleate embryo sac. The egg apparatus and polar nuclei are comparatively small and inconspicuous as compared to the antipodal cells. Usually, both the synergids degenerate soon after fertilization, but sometimes one of them persists. The polar nuclei always lie in close proximity to the antipodal cells and fuse before fertilization. The antipodal cells are large and vesicular. They continue to enlarge after fertilization, persist up to the heart-shaped stage of the proembryo and serve a haustorial role.

Double fertilization occurs and the primary endosperm nucleus divides much earlier than the oospore. The endosperm is of the nuclear type. Wall formation is centripetal and while the proembryo is still undifferentiated, the cellular endosperm fills the entire embryo sac.

The first division of the zygote is transverse. Both the cells of the two-celled proembryo divide transversely or vertically, or the basal cell may divide by an oblique transverse wall and the terminal cell by a vertical wall, or the basal divides by an obliquely vertical wall and the terminal cell by an obliquely transverse wall.

Polyembryony has been reported. The additional proembryo develops from one of the persisting synergids.

The black stony seed contains copious endosperm and comparatively small embryo which sometimes shows unequal cotyledons. Both endosperm and embryo contain abundant oil globules. The seed also shows an epistase at the micropylar end and a laterally placed hypostase at the chalazal end.

The seed coat comprises three layers, the two outer thickened ones are derived from the outer integument, and the innermost which is slightly cutinized along the inner surface is the persistent layer of the inner integument.

It gives me great pleasure to express my sincere thanks to Professor P. Maheshwari and Dr. B. M. Johri for their valuable suggestions and keen interest during the course of this investigation.

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SOME OBSERVATIONS ON ENDOSPERM DEVELOPMENT IN THE CUCURBITACEAE

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Introduction

The embryology and seed structure of the Cucurbitaceae have attracted much attention (*see* Kirkwood, 1904, 1906; Kratzer, 1918; Netolitzky, 1926; Banerji & Das, 1937; Souèges, 1939; Bahadur Singh, 1952, 1953). Among special features may be mentioned the persistent pollen tube which in some cases branches after reaching the ovule and seems to play an important role in the nutrition of the embryo, and the three anatomically characteristic layers of the seed coat produced by the epidermis of the outer integument. The nature of the placentae and the type of placentation also present interesting problems (*see* Puri, 1954).

The present investigation on the Cucurbitaceae was begun in the year 1952 at the suggestion of Professor P. Maheshwari. Whole mounts of endosperm of *Momordica charantia* L. revealed a prominent chalazal caecum. A perusal of the literature indicated that this structure had either been missed or only incompletely understood by the previous workers. Several other members of the family were, therefore, examined and an endosperm haustorium was noticed in the following species: *Coccinia indica* W. & A. (syn. *Cephalandra indica* Naud.), *Luffa aegyptiaca* Mill.[1] (syn. *L. cylindrica* Roem.), *L. acutangula* Roxb., *Cucumis melo* L., *C. melo* L. var. *utilissimus* Roxb., *C. melo* L. var. *momordica* Roxb., *Cucumis sativus* L., *Citrullus vulgaris* Schrad., *C. fistulosus* Stocks, *C. colocynthis* Schrad., *Trichosanthes anguina* L., *T. cucumerina* L., *T. bracteata* Voigt., *Lagenaria vulgaris* Ser., *Cucurbita moschata* Duch., *Actinostemma tenerum* Griff., *Benincasa hispida* Cogn. and *Melothria maderaspatana* Cogn. (syn. *Mukia scabrella* Arn.). Of the species examined only *Blastania garcini* Cogn. did not show any indication of haustorium formation.

Three brief notes (Chopra, 1953a,b, 1954) have already been published on this subject. Recently, Dalbir Singh (1955) has reported a chalazal endosperm haustorium in *Cucumis melo* L. var. *pubescens* Willd., and Weiling & Schagen (1955) have given an account of this structure in five species of *Cucurbita*, viz., *C. mixta* Pang., *C. pepo* L., *C. moschata* Duch., *C. ficifolia* Bouché, and *C. maxima* Duch. Johri & Roy Chowdhury (1955) have studied in detail the endosperm development in *Citrullus colocynthis* Schrad. and *Melothria maderaspatana* Cogn.

This paper gives a comparative account of the endosperm development, with special reference to the structure and behaviour of the chalazal haustorium, in *Citrullus fistulosus, Coccinia indica, Momordica charantia, Lagenaria vulgaris, Cucumis sativus* and *Trichosanthes anguina. Blastania garcini* has also been studied.

Material and Methods

The material of *Citrullus fistulosus, Momordica charantia, Lagenaria vulgaris, Cucumis sativus* and *Trichosanthes anguina* was collected from plants cultivated in the Delhi University Botanical Garden, that of *Coccinia indica* from wild plants growing in the University campus, and of

1. Regarding my observations on this species Weiling & Schagen (1955; p. 8) write: "Ein sehr kurzes und dünnes Haustorium findet sich bei *Luffa aegyptiaca* Mill. Diese Art weist wie *Cucurbita maxima* im chalazalen Teil des Endosperms Zotten auf, die jedoch im Gegensatz zu dieser Art aus mehreren Zellen bestehen (Chopra, 1954)." While they have correctly referred to the multicelled mounds of *Luffa aegyptiaca*, the degenerating haustorium sketched in Fig. 8 has been mistaken for a healthy one.

Blastania garcini from Agra. The period of collection extended from July to October, 1952. Formalin-acetic-alcohol was used as the fixative and 70 per cent ethyl alcohol for preservation.

Dissections were made under a binocular stereo-microscope with the help of sharp needles. A median longitudinal slit was made in the ovule from below upwards, first through the integuments and then through the nucellus. By this method it was possible to dissect out a close series of developmental stages from the fertilized embryo sac onwards. The endosperms were stained in a drop of acetocarmine and then mounted in a mixture of the stain and 50 per cent glycerine. After 24-48 hours the cover glass was sealed with thin canada balsam. Such slides, prepared about 3 years ago, are still in good condition.

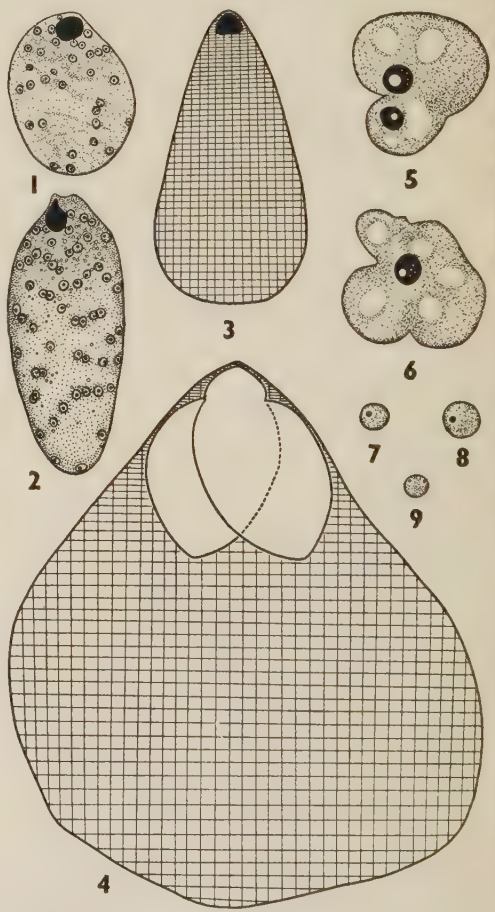
In *Trichosanthes anguina* fresh material from the field was also studied. In some of these preparations neutral red was used for staining.

Microtome sections were cut 12-30 μ thick and stained with iron-haematoxylin as well as safranin and fast green. Sections failed, however, to give a complete picture of the haustorium and stress was, therefore, laid on the study of whole mounts. Free-hand sections of mature seeds were cut with a view to determine the fate of the nucellus and the endosperm.

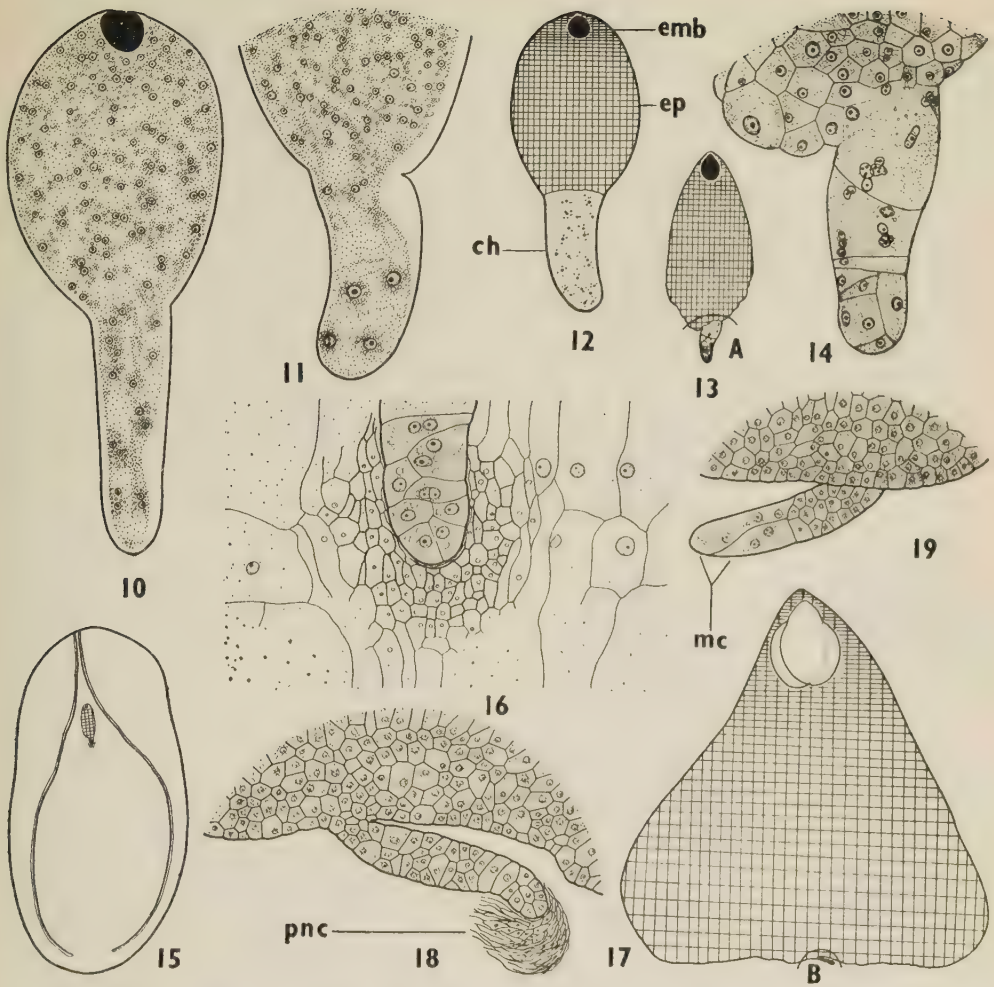
Observations

The endosperm is of the Nuclear type. The nuclei become distributed in a peripheral layer of cytoplasm, and the centre is occupied by a large vacuole. The embryo sac increases in size and elongates considerably in all cases except *Blastania garcini* which, as already stated, lacks the chalazal haustorium (Figs. 1, 2, 20, 31). Subsequently, the upper part of the embryo sac broadens while the lower remains narrow (Fig. 10) and grows towards the chalaza. In *Cucumis sativus* and *Trichosanthes anguina* its growth is very rapid. Fig. 46 shows free nuclear endosperm of *C. sativus* in which the upper part of the embryo sac is only 200 μ in length, while its chalazal extension has become 1,200 μ long. The tip of the haustorium in these species frequently reaches almost to the base of the nucellus at an early stage of development (Fig. 47). In other plants like *Citrullus fistulosus* and *Momordica charantia* the haustorium remains fairly short (Figs. 15, 29). As development proceeds, starch grains so commonly met with in the mature embryo sac, are gradually consumed. In *Blastania garcini* and *Citrullus fistulosus*, however, they may persist up to a very late stage (Fig. 19).

While the rest of the nucellar cells enlarge considerably and undergo gradual



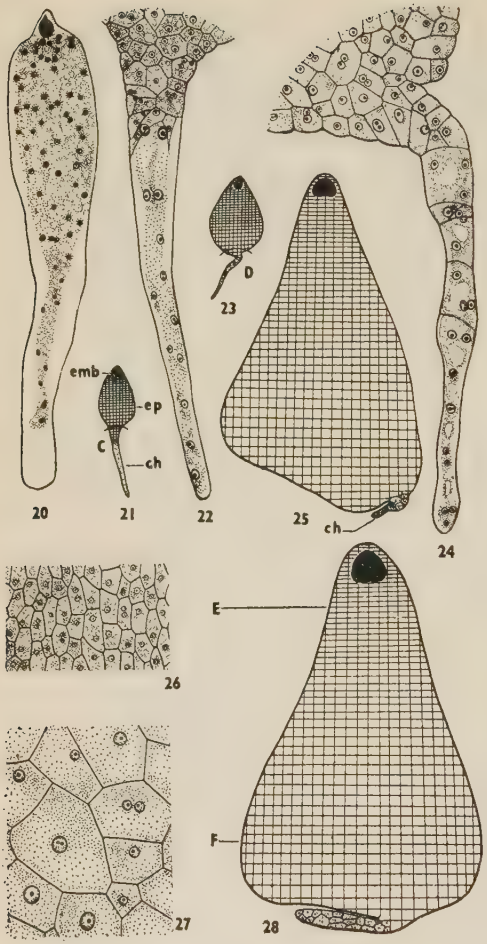
Figs. 1-9—*Blastania garcini*. Figs. 1-4. Different stages of endosperm development; note the absence of the chalazal haustorium. Figs. 1,2. × 108; Figs. 3, 4. × 20. Figs. 5, 6. Nuclei of central endosperm cells. × 400. Figs. 7-9. Same, from peripheral cells. × 400. (All figures from whole mounts; Figs. 3, 4 diagrammatic).



FIGS. 10-19 — *Citrullus fistulosus* (*ch*, chalazal haustorium; *emb*, embryo; *ep*, endosperm proper; *pnc*, pocket of nucellar cells). Fig. 10. Nuclear endosperm. × 108. Fig. 11. Lower part of an embryo sac with a short haustorium having only four large nuclei. × 108. Fig. 12. Cellular endosperm with the coenocytic haustorium. × 92. Fig. 13. The haustorium has been partitioned into multinucleate cells. × 24. Fig. 14. Enlargement of part *A* of Fig. 13. × 108. Fig. 15. L.s. ovule, showing the extent of haustorial development. × 11. Fig. 16. L.s. lower portion of haustorium with surrounding nucellar cells. Note the smaller thick-walled cells and the large disintegrating cells around them. × 208. Fig. 17. Endosperm with the cellular haustorium pressed to its base. × 11. Fig. 18. Enlargement of part *B* of Fig. 17; note the pocket of nucellar cells at the tip of the haustorium. × 92. Fig. 19. Lower portion of endosperm proper with the haustorium, the apical part of which still retains the multinucleate cells (*mc*). × 92. (All figures except 15, 16 from whole mounts; Figs. 12, 13, 15, 17 diagrammatic; hypostase drawn only in Figs. 16 and 18).

disorganization, the cells of the hypostase remain small and become slightly thick-walled (Fig. 16). The latter forms a cup-like structure which persists at the tip of the haustorium in *Citrullus fistulosus* (Fig. 18). A similar 'pocket' of nucellar cells has been reported in *C. citrullus* (Kirkwood, 1904) and *C. colocynthis* (Johri & Roy Chowdhury, 1955).

Wall formation in the endosperm is initiated in the vicinity of the embryo.



FIGS. 20-28 — *Coccinia indica* (*ch*, chalazal haustorium; *emb*, embryo; *ep*, endosperm proper). Fig. 20. Enlarged embryo sac with free endosperm nuclei. × 108. Fig. 21. The upper part of the embryo sac has become cellular, while the haustorium is still coenocytic. × 24. Fig. 22. Enlarged view of part *C* of Fig. 21. × 108. Fig. 23. The haustorium has become partitioned. × 24. Fig. 24. Part *D* of Fig. 23 enlarged to show multinucleate cells in the haustorium. × 108. Fig. 25. Endosperm with a laterally situated haustorium. × 24. Figs. 26, 27. Enlarged portions of endosperm proper at the levels *E* and *F* marked in Fig. 28. × 108. Fig. 28. Endosperm with a cellular haustorium lying close to its base. × 24. (All figures from whole mounts; Figs. 21, 23, 25, 28 diagrammatic).

It progresses downwards and centripetally (Figs. 30, 32, 33) till the whole of the upper broader part becomes cellular, while the chalazal extension remains free nuclear[2] (Figs. 12, 21, 35, 39, 48, 51). The cellular endosperm, which may be called the 'endosperm proper', increases in size both by cell division and cell enlargement. Some of the cells show a multinucleate condition (Figs. 22, 30, 40). In later stages the lower portion of the endosperm becomes broader than the upper (Figs. 3, 4, 17, 28, 37, 44, 53) and shows larger cells (Fig. 27). The cells in the micropylar region remain small and have denser contents (Fig. 26).

The general form of the haustorium varies in different species, and also to some extent in the same species (Figs. 12, 22, 34, 40, 42, 43, 48, 50, 51). Its maximum length and breadth in different regions is given in the following table:

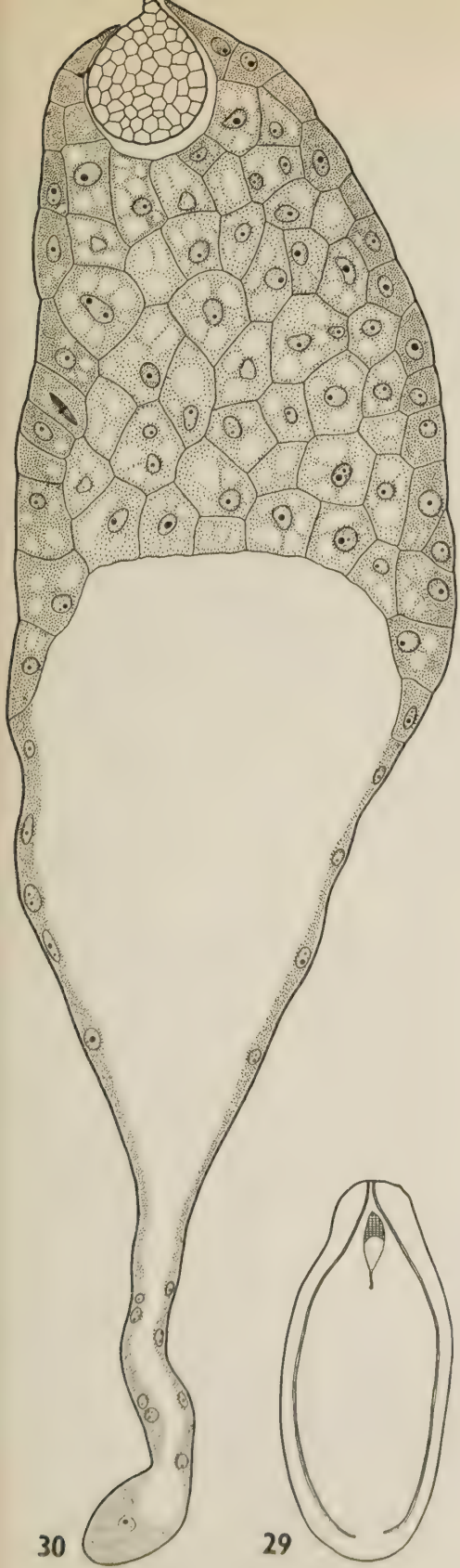
Name of Plant	Chalazal Endosperm Caecum	
	Length (in μ)	Breadth (in μ)
Citrullus fistulosus	333	60-120
Coccinia indica	645	40-100
Momordica charantia	740	60-100
Lagenaria vulgaris	1,673	40-100
Cucumis sativus	5,250	35-100
Trichosanthes anguina	6,317	51-60

In *Cucumis sativus*, below the endosperm proper, there is a prominent bulbous free nuclear portion (Figs. 48, 50) whose diameter varies from 320-460 μ. The tip of the haustorium in this plant is also often markedly swollen (Figs. 48, 49) and may be 200-300 μ broad.

The haustorium usually occupies a central position, but in *Coccinia indica* it may come to lie laterally (Fig. 25) due to asymmetrical growth of the endosperm proper.

As a rule, the haustorium contains dense cytoplasm, several free nuclei and in some cases abundant starch grains. The nuclei often vary in size and may aggregate in groups of 3-10 (Figs. 10, 22, 34, 40, 42, 43, 46, 51, 52). In one preparation of *Citrullus fistulosus* the haustorium was rather short and contained only 4 nuclei, which were much larger than those in the upper part (Fig. 11).

2. The mode of wall formation has been observed in *Citrullus fistulosus*, *Momordica charantia* and *Cucumis sativus*.



A study of the fresh seeds of *Trichosanthes anguina* showed protoplasmic streaming in the coenocytic appendage as also observed by Kausik (1941) in *Grevillea robusta* (Proteaceae), Anantaswamy Rau (1953) in *Desmodium triflorum* (Papilionaceae), and Goldberg (1941) in the giant haustorial cell of *Peltandra virginica* (Araceae).

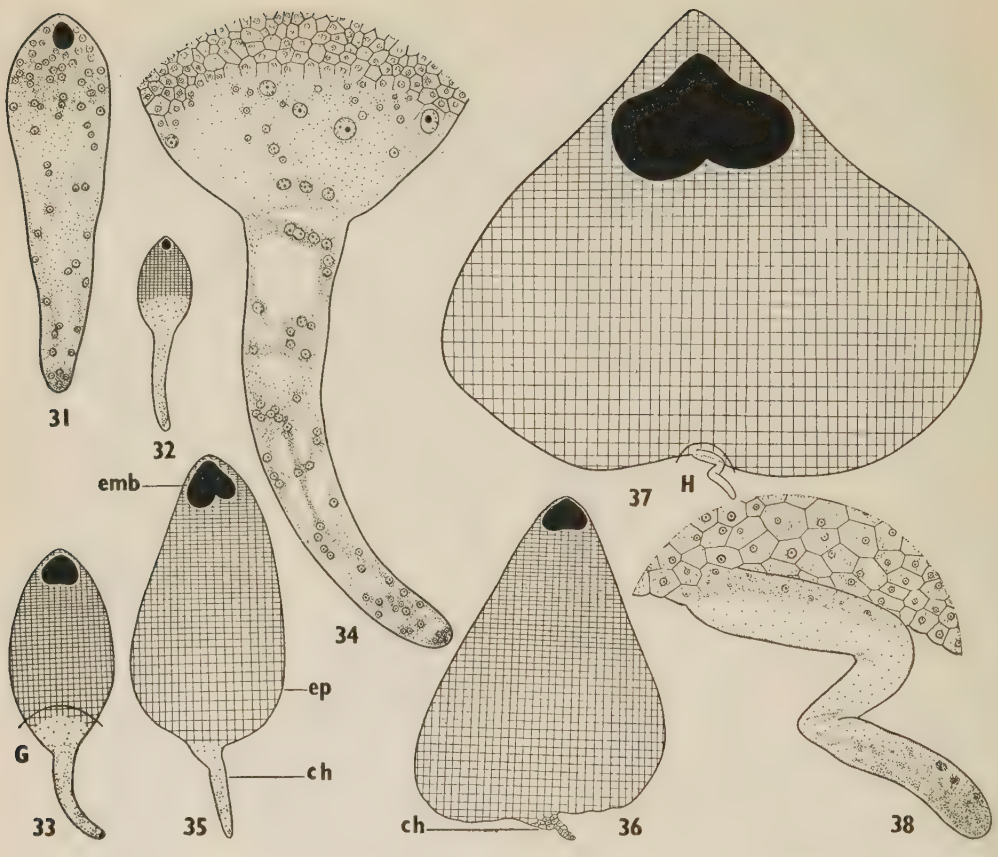
In *Citrullus fistulosus* and *Coccinia indica* the haustorium gets partitioned in such a way that cells of different sizes containing a variable number of nuclei are formed (Figs. 13, 14, 23, 24). Nuclear fusions have been observed in some of these cells (Fig. 14). Subsequently, more walls are laid down giving rise to a multiseriate structure (Figs. 18, 28). An interesting variation is the retention of multinucleate compartments in a portion of the haustorium of *Citrullus fistulosus* (Fig. 19); in *Coccinia indica* its apical half or even two-thirds may remain coenocytic. In *Momordica charantia*, *Lagenaria vulgaris*, *Cucumis sativus* and *Trichosanthes anguina* the haustorium remains in the free nuclear condition throughout (Figs. 35. 41. 50, 53). Some cases of wall formation have, however, been observed in *Momordica charantia* (Fig. 36). A synopsis of the behaviour of the haustorium is given below:

Name of the Plant	Haustorium
Citrullus fistulosus	Always becomes cellular
Coccinia indica	Mostly becomes cellular
Momordica charantia	Mostly remains coenocytic
Lagenaria vulgaris	Always remains coenocytic
Cucumis sativus	do
Trichosanthes anguina	do

Usually, the haustorium remains quite healthy up to the heart-shaped stage of the embryo after which its activity declines. When coenocytic, it becomes somewhat folded due to the downward extension of the endosperm proper

←—

Figs. 29, 30 — *Momordica charantia*. Fig. 29. Diagram l.s. ovule showing the extent of haustorial development. × 13. Fig. 30. Endosperm from Fig. 29 enlarged to show the mode of wall formation (reconstructed). × 257.



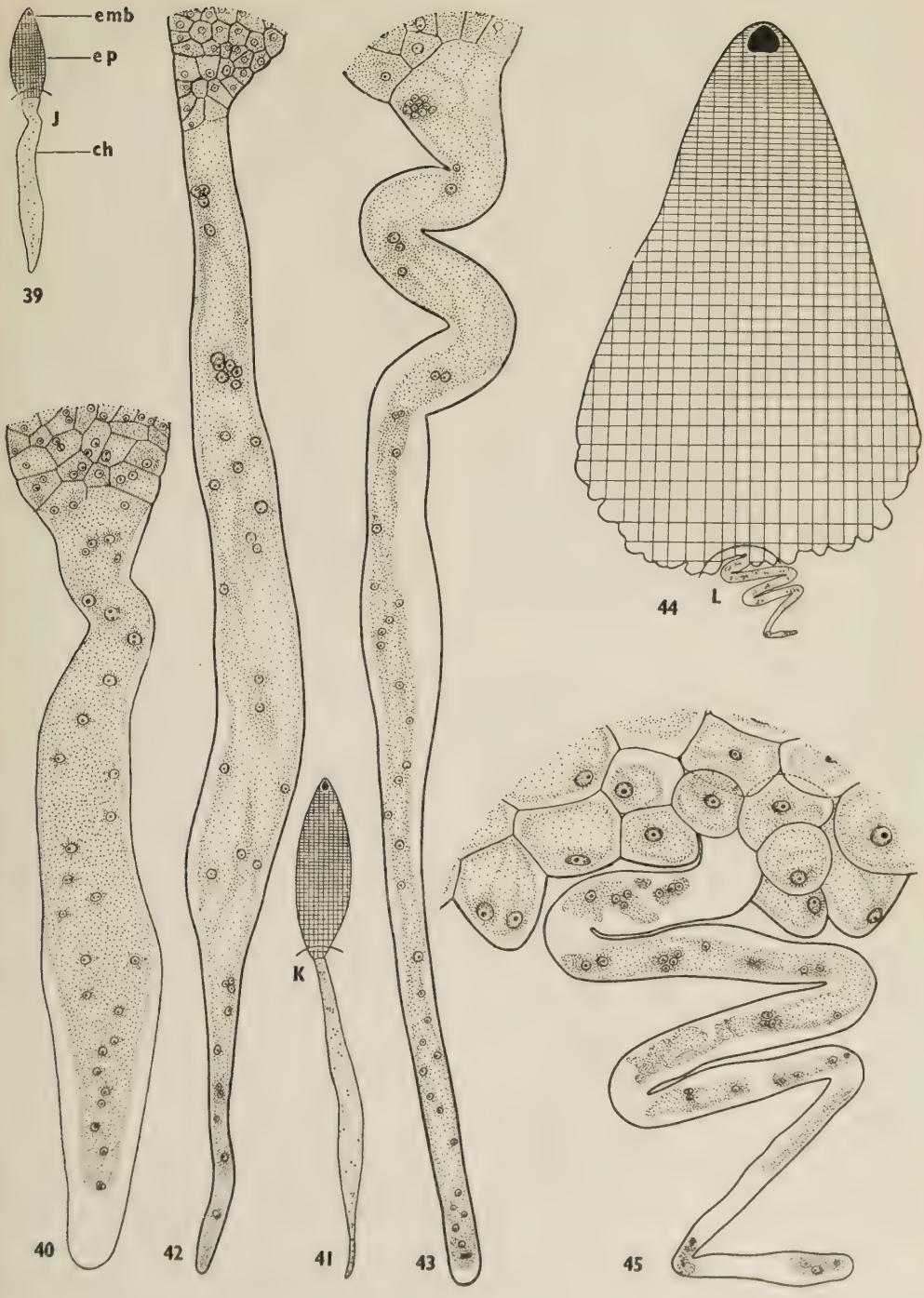
FIGS. 31-38 — *Momordica charantia.* (*ch*, chalazal haustorium; *emb*, embryo; *ep*, endosperm proper). Fig. 31. Embryo sac with endosperm nuclei. × 103. Figs. 32, 33, 35. Diagrams showing progressive wall formation. × 19. Fig. 34. Enlargement of portion *G* of Fig. 33. × 103. Fig. 36. Endosperm with the chalazal haustorium which has become cellular (diagrammatic). × 19. Fig. 37. Endosperm with slightly folded haustorium (diagrammatic). × 23. Fig. 38. Enlargement of part *H* of Fig. 37. × 103. (All figures from whole mounts).

(Figs. 37, 38, 44, 45). Its contents gradually degenerate (Figs. 38, 45) and eventually it completely collapses so that in older stages even its remnants cannot be made out. In the species where it becomes cellular it does not degenerate, but bends near its base and comes to lie close to the endosperm proper (Figs. 17, 18, 19, 28).

As the activity of the main haustorium decreases, the peripheral cells in the basal region of the cellular endosperm often bulge out so that its outer surface becomes

→

FIGS. 39-45 — *Lagenaria vulgaris.* (*ch*, chalazal haustorium; *emb*, embryo; *ep*, endosperm proper). Figs. 39, 41. Cellular endosperm with coenocytic chalazal haustorium. × 29. Figs. 40, 42. Enlargements of portions marked *J* and *K* in Figs. 39 and 41 respectively. × 133. Fig. 43. A coenocytic haustorium. Note the nuclear aggregation in its upper part. × 133. Fig. 44. Endosperm proper at the heart-shaped stage of the embryo. Note the bulging marginal cells in the lower portion and the folded haustorium. × 24. Fig. 45. Enlargement of part *L* of Fig. 44 to show haustorium and papillate cells at the margin of the endosperm proper. × 133. (All figures from whole mounts; Figs. 39, 41, 44 diagrammatic).



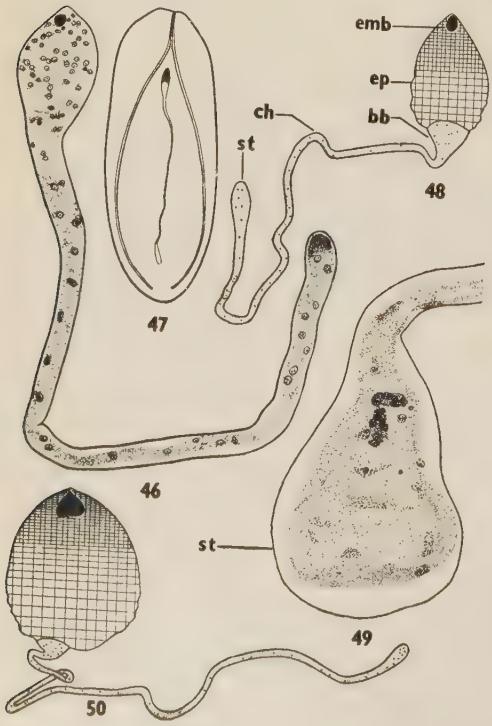
FIGS. 39-45.

wavy (Figs. 44, 50, 53). This feature is well marked in *Lagenaria vulgaris*, *Cucumis sativus* and *Trichosanthes anguina* in which these cells enlarge, get rather loosely arranged and form papillate outgrowths and reaches up to the base of the nucellus. The following table gives the length and breadth of the endosperm proper at different stages of embryo development in *Coccinia indica* and *Momordica charantia*:

Stage of Embryo	Endosperm of *Coccinia indica*		Endosperm of *Momordica charantia*	
	Length (in μ)	Breadth (in μ)	Length (in μ)	Breadth (in μ)
Early globular	422	220	600	373
Late globular	1,220	712	1,356	712
Heart-shaped	2,407	1,610	2,120	1,695
Cotyledons differentiated	4,576	3,051	3,800	4,300

(Fig. 45). They show dense cytoplasm and larger nuclei which suggest their haustorial role, and may thus be termed ' secondary haustoria '. In *Luffa aegyptiaca* (Chopra, 1954) such cells divide to form characteristic ' mounds '.

After the heart-shaped stage of the embryo the endosperm grows very rapidly



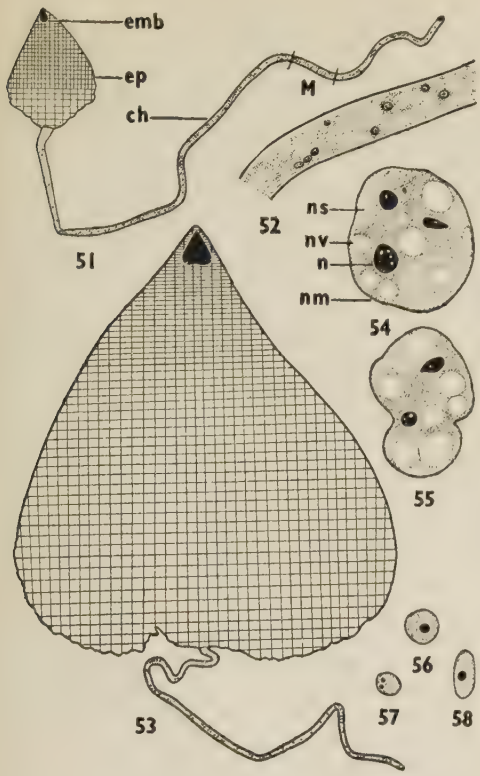
Figs. 46-50.

The central cells of the endosperm proper enlarge appreciably especially along the longitudinal axis of the seed. Their nuclei also increase greatly in size (Figs. 5, 6, 54, 55) measuring from 44-86 μ. The peripheral cells, on the other hand, remain small and so also their nuclei (Figs. 7-9, 56-58), the latter having a diameter of only 8-15 μ. Many of the nuclei become multinucleolate, the number of nucleoli varying from 2 to 4. The larger nuclei as well as their nucleoli become irregular in shape and develop vacuoles (Figs. 5, 6, 54, 55). Similar, but much larger (20-200 μ in diameter) nuclei have been reported in *Echinocystis macrocarpa* by Scott (1944, 1953). She studied them in the living condition under phase microscope and observed Brownian movement and streaming of particles, formation, pulsation and translocation of nuclear vacuoles, and amoeboid changes of form.

The endosperm develops at the expense of the nucellar tissue, of which only a few compressed layers persist and form a thin covering over the embryo. The outer tangential and radial walls of the nucellar

←

Figs. 46-50 — *Cucumis sativus*. (*bb*, bulbous base; *ch*, chalazal haustorium; *emb*, embryo; *ep*, endosperm proper; *st*, swollen tip). Fig. 46. Nuclear endosperm with a long chalazal haustorium. × 91. Fig. 47. L.s. ovule; the tip of the haustorium has reached almost to the base of the nucellus. × 9. Figs. 48, 50. Cellular endosperm with the coenocytic haustorium. × 20. Fig. 49. Apical portion of a haustorium enlarged to show the swollen tip. × 91. (All figures except 47 from whole mounts; Fig. 47 reconstructed).



FIGS. 51-58 — *Trichosanthes anguina*. (*ch*. chalazal haustorium; *emb*, embryo; *ep*, endosperm proper; *n*, nucleolus; *nm*, nuclear membrane; *ns*, nuclear sap; *nv*, nuclear vacuole). Fig. 51. Cellular endosperm with coenocytic haustorium. × 22. Fig. 52. Enlargement of portion marked *M* in Fig. 51. × 100. Fig. 53. Later stage in development of endosperm. The peripheral cells in the basal region have bulged out and the haustorium is coenocytic. × 22. Figs. 54, 55. Nuclei from central endosperm cells. × 365. Figs. 56-58. Same, from peripheral cells. × 365. (All figures from whole mounts; Figs. 51, 53 diagrammatic).

epidermis become thickened. The endosperm is, in turn, consumed by the rapidly growing embryo. Its cells become flaccid and the walls gradually collapse. In the mature seeds only the outermost layer of the endosperm persists along with some degenerated remains especially between and near the tips of the cotyledons.

Discussion

The general features of endosperm development in the Cucurbitaceae have been known for some time, but information about the structure and behaviour of the chalazal haustorium was very meagre. Some earlier workers figured a tube-like prolongation at the lower end of the endosperm: Brongniart (1827) in *Pepo macrocarpus*, Mirbel (1829) in *Cucumis leucantha*, and Amici (1842; quoted in Facchini, 1844) in *Cucurbita pepo*. Garreau (1849) drew a similar structure in *Cucumis sativus*, but seems to have missed it in *Momordica elaterium*. Hofmeister (1849) described certain features of the endosperm in *Cucurbita*, *Sicyos* and *Ecballium*, but did not record any chalazal caecum. Kirkwood (1904) studied the endosperm development in 15 species, but reported the presence of a haustorium only in *Apodanthera undulata*, *Fevillea cordifolia* and *Citrullus citrullus*. In some others, he did not fully understand its morphology; about *Momordica charantia* he writes: "*Sometimes*[3] a thin line of cytoplasm may be seen penetrating the tissue towards the chalaza." In *Melothria pendula*, *Cucumis myriocarpus*, *Benincasa hispida* and *Sicyos angulata* also, he describes it as a delicate strand of endosperm. No mention is made of the haustorium in *Bryonopsis laciniosa erythrocarpa*, *Trichosanthes anguina*, *Luffa acutangula*, *Lagenaria lagenaria*, *Cucurbita pepo*, *Micrampelis lobata* and *Cyclanthera explodens*.

Scott (1944) reports a non-cellular caecum at the distal end of the endosperm in *Echinocystis macrocarpa*. Bahadur Singh (1952) did not refer to any such structure in *E. wrightii*, but recently Weiling & Schagen (1955) have observed a short cone-shaped haustorium in this species. In a later paper on the structure and development of the seeds in *Bryonia dioica*, *Cucumis melo*, *Cucurbita pepo*, *Cyclanthera explodens*, *Ecballium elaterium*, *Lagenaria vulgaris*, *Luffa cylindrica*, *Sicyos angulata*,[4] and *Trichosanthes anguina* Bahadur Singh (1953) writes: "The endosperm which becomes enormous, begins development with free nuclear divisions but later becomes cellular and

3. Italics mine.

4. Bahadur Singh (1953) refers to this plant as *Sicyos angulatus* L., but the correct specific name is *S. angulata* L. (Index Londinensis, Vol. VI p. 114; Index Kewensis, Vol. II p. 896).

reaches down almost to the base. It is ultimately nearly consumed by the growing embryo." This statement indicates that in these species also he did not observe any chalazal coenocytic prolongation, nor did Chakravorti (1947) make any mention of it in *Coccinia indica*.

Of the various genera and species in which the endosperm haustorium had been missed earlier, I have observed it in *Coccinia indica, Lagenaria vulgaris, Trichosanthes anguina, Cucumis melo, Luffa cylindrica, L. acutangula, Momordica charantia, Benincasa hispida* and *Melothria maderaspatana*. Additional plants in which it has been noticed are: *Citrullus fistulosus, C. vulgaris, C. colocynthis, Trichosanthes cucumerina, T. bracteata, Actinostemma tenerum, Cucurbita moschata, Cucumis melo* var. *utilissimus*, and *C. melo* var. *momordica*.

The main reason why the haustorium had been either missed or only incompletely understood is due to the exclusive use of microtome sections which fail to give an adequate picture of this structure. It was chiefly with the help of dissected whole mounts that the author was able to observe it and study its behaviour in some detail (*see* also Chopra, 1953a,b, 1954).

Some other instances where the dissection method has led to a correct understanding of such haustorial processes are the Proteaceae[5] (Kausik, 1938, 1939, 1942), the Leguminosae[5] (Anantaswamy Rau, 1950, 1951a, b, 1953; Dnyansagar, 1954 a, b, c) and the Santalaceae (Paliwal, 1953; Maheshwari & Ghosh, 1955; Ghosh, 1955). Many years ago, Buchholz (1938) advocated a similar technique for studying the tortuous suspensor systems of coniferous embryos.

In the Cucurbitaceae, the endosperm haustorium develops at the distal end of the embryo sac during the post fertilization stages. To begin with it is coenocytic in all the species, but later on in some cases, e.g., *Citrullus citrullus* (Kirkwood, 1904), *C. fistulosus* (present work), and *C. colocynthis* (Johri & Roy Chowdhury, 1955), it enters into the cellular phase, while in others, e.g., *Apodanthera undulata* (Kirkwood, 1904), *Luffa aegyptiaca* (Chopra, 1954), *Cucumis sativus, Lagenaria vulgaris, Trichosanthes anguina* (present work), *Cucumis melo* var. *pubescens* (Dalbir Singh, 1955), five[6] species of *Cucurbita* (Weiling & Schagen, 1955) and *Melothria maderaspatana* (Johri & Roy Chowdhury, 1955), it retains its free nuclear condition throughout. *Coccinia indica* and *Momordica charantia* show a variable behaviour. In *Coccinia* the haustorium usually becomes cellular, but sometimes its apical half or even more remains coenocytic. In *Momordica*, on the other hand, the haustorium as a rule retains its free nuclear condition, although occasionally wall formation also occurs.

The permanent coenocytic condition of the appendage seems to be more suited for a haustorial function, as it allows for free cytoplasmic movement which is not possible when it gets partitioned into smaller compartments. Its length is also directly related to its absorptive surface, and in plants like *Cucumis sativus* and *Trichosanthes anguina* where the tip of the haustorium frequently reaches the chalazal region, it forms a direct channel for the transportation of food materials[7].

The haustorium transfers the food materials from the nucellar tissue to the endosperm proper, which supplies them to the embryo. The haustorium is healthy and active during early stages of embryo development, but after the heart-shaped stage it either disorganizes (when coenocytic), or it becomes pressed against the base of the endosperm proper (when cellular).

A feature of considerable interest is the bulging out of the peripheral basal cells of the endosperm proper, which in some species are quite prominent and have been referred to as ' secondary haustoria '. In such cases, therefore, the whole of outer surface in the basal part becomes active and causes a quick dissolution of the remaining nucellar cells. It may be mentioned here that *Citrullus fistulosus, Coccinia indica* and *Momordica charantia* which have a short chalazal haustorium

5. The origin, structure and fate of the endosperm haustorium in these two families is essentially similar to that in the Cucurbitaceae; a detailed comparison would be quite interesting.

6. *Cucurbita mixta, C. pepo, C. ficifolia, C. maxima* and *C. moschata*.

7. Weiling & Schagen (1955) report a similar phenomenon in some of the *Cucurbita* spp. In *C. ficifolia* the haustorium attains a length of as much as 12,000 microns.

are also devoid of any well developed 'secondary haustoria'.

Blastania garcini is quite peculiar in that it lacks the main chalazal haustorium as well as the 'secondary haustoria'. The general development of the endosperm, however, follows the same plan as in the other species.

The number of plants so far investigated is rather limited and most of them belong to the tribe Cucurbiteae. Before any taxonomic significance can be attached to the haustorium, it would be necessary to make a detailed study of its structure and behaviour in all the five tribes: Fevilleae, Melothrieae, Cucurbiteae, Sicyoideae and Cyclanthereae. Further, it would be worthwhile to reinvestigate the families allied to the Cucurbitaceae to see if they have any such structure.

Summary

A chalazal endosperm haustorium has been observed in 11 genera and 17 species of the Cucurbitaceae. It is not formed in *Blastania garcini*. Detailed investigation has been made of the endosperm development in *Blastania garcini*, *Citrullus fistulosus*, *Coccinia indica*, *Momordica charantia*, *Lagenaria vulgaris*, *Cucumis sativus* and *Trichosanthes anguina*.

The endosperm is of the Nuclear type. During early post-fertilization stages the lower part of the embryo sac grows downwards into a long tubular process. After some time wall formation occurs in the upper part of the embryo sac, which is then referred to as the 'endosperm proper'. The chalazal coenocytic haustorium elongates further and in some cases its tip frequently reaches down to the chalazal region. Its maximum length varies from 333 μ in *Citrullus fistulosus* to over 6,000 μ in *Trichosanthes anguina*. It contains dense cytoplasm and several nuclei which often aggregate in groups of 3-10.

Subsequently, the haustorium becomes cellular in *Citrullus fistulosus*, while in *Lagenaria vulgaris*, *Cucumis sativus* and *Trichosanthes anguina* it retains its free nuclear condition throughout. *Coccinia indica* and *Momordica charantia* show a variable behaviour.

As a rule, the haustorium functions actively up to the heart-shaped stage of the embryo after which its activity declines. When coenocytic, it simply disorganizes, but when cellular it becomes pressed to the base of the endosperm proper.

The endosperm proper increases greatly in size. Its lower part becomes much broader than the upper and the peripheral cells of this region often bulge out and serve as 'secondary haustoria'. The nuclei of the central endosperm cells enlarge and have a diameter ranging from 44-86 μ. They become multinucleolate and acquire an irregular outline. Vacuoles appear in these nuclei as well as in their nucleoli.

The nucellus is gradually disorganized by the developing endosperm proper and its chalazal haustorium. Only a few of its compressed layers persist, which form a thin covering over the embryo. The rapidly growing embryo consumes the endosperm tissue, which ultimately collapses; the mature seeds are non-endospermic.

It gives me great pleasure to express my deep gratitude to Professor P. Maheshwari and Dr. B. M. Johri for their keen interest and valuable suggestions during the course of this study.

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ANATOMICAL STUDIES ON THE SHOOT APICES OF SOME PARASITIC AND SAPROPHYTIC ANGIOSPERMS

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Introduction

The importance of nutrition as a factor affecting plant form has long been recognized, and has been the subject both of experimental investigations and of some theoretical consideration (e.g. Allsopp, 1953a, 1953b, 1954; Goebel, 1908; Wardlaw, 1945, 1952; Wetmore, 1950, 1954). Since the assumption of form takes place in the embryonic and meristematic regions of the plant, it is in the apical meristems that any developmental peculiarity due to anomalous nutrition may be expected to occur. The present investigation was therefore undertaken as a preliminary exploration to discover whether the shoot apices of plants with a parasitic or saprophytic habit of growth exhibit any special interest as prospective material for morphogenetic studies. It is noteworthy that while many studies of the anatomy of parasitic plants have been made in the past, few have been concerned with the meristematic regions of the adult plant, although some embryological work has been carried out.

Having regard to the time and material available for the present study, it was not possible to attempt a complete anatomical investigation of the species examined, related to growth and ontogenetic development. The observations recorded below are therefore essentially a preliminary survey and do not claim to give a complete developmental account of the apices studied. They are, however, sufficient to justify the conclusion that the anomalous mode of nutrition of these plants has not, in general, caused any unusual developments in their apical organization as compared with that of autotrophic plants.

Throughout this investigation the tunica-corpus concept has been used in describing cellular segmentation at the apex. The tunica has been variously interpreted in the past; in the present account it comprises those layers of the apical meristem in which no or very few periclinal divisions occur (except in leaf or bud formation) in all the apices of that species observed. Other stratified layers in which more frequent periclinal divisions occur are considered to form part of the corpus. The number of stratified layers in the meristem is known to fluctuate due to season (Esau, 1953; Reeve, 1948), to stages in ontogeny (Chakravarti, 1953; Foster, 1949), and to the stage of the plastochrone reached (Esau, 1953; Reeve, 1948; Sussex, 1952).

Wardlaw (1952, 1953a) has directed attention to the lack of precise information on the supply of nutrients to the shoot apex and, drawing on experimental evidence in ferns and angiosperms, has speculated upon the probable distribution of nutrients in the apex. Emphasizing the necessity for a more dynamic approach to the problems of apical organization, he points out that both a differential supply of nutrients to the various layers of the apex, and differential rates of growth of the various layers within the apex, could account for some of the variations in histological organization in the apices of vascular plants (Wardlaw, 1951, 1953a). It is therefore clearly of interest to enquire into the possible effects of unusual methods of nutrient supply to the whole shoot.

Material and Methods

Shoot apices of various parasitic and saprophytic angiosperms were either collected by the writer or received from

various donors. The material was therefore fixed in a variety of fixing soultions, which are listed below; in some cases their constitution was not known in detail.

Aeginetia indica	Formalin-acetic-alcohol (Johansen, 1940)
Balanophora sp.	Acetic alcohol
Cassytha filiformis	Acetic alcohol
Cuscuta americana	70% alcohol
C. europaea	Formalin-acetic-alcohol
Lathraea clandestina	Formalin-acetic-alcohol
Loranthus globiferus	Formalin-alcohol (McLean & Cook, 1941)
Neottia nidus-avis	Chromacetic solution (Chamberlain, 1926) and Formalin-acetic-alcohol
Orobanche hederae	Formalin-acetic-alcohol
Phoradendron flavescens	Formalin-acetic-alcohol (70%)
Viscum album	Formalin-acetic-alcohol

Serial sections 6-10 μ thick were stained in Delafield's haematoxylin and safranin (Chamberlain, 1926), Johansen's (1940) quadruple stain — safranin, methyl violet, orange G and fast green — and Sharman's (1943) tannic acid with iron alum, orange G and safranin. Usually the orange G was omitted from the latter combination.

Terminology

Visible leaf primordia are called P_1, P_2, etc., P_1 being the youngest, and the positions of prospective primordia are called I_1, I_2, etc., I_1 being the next to arise (Snow & Snow, 1931).

The term *prestelar tissue* (Wardlaw, 1950) is used for the undifferentiated vascular tissue. The criteria adopted in ascertaining the direction of differentiation of the prestelar tissue were those of Esau (1954): differentiation was considered to be acropetal if no discontinuity was observed between prestelar tissue and differentiated vascular tissue, and if the prestelar tissue became progressively more distinct in a basipetal direction.

Anatomical Observations

NEOTTIA NIDUS-AVIS (L.) L. C. RIGH. (ORCHIDACEAE)

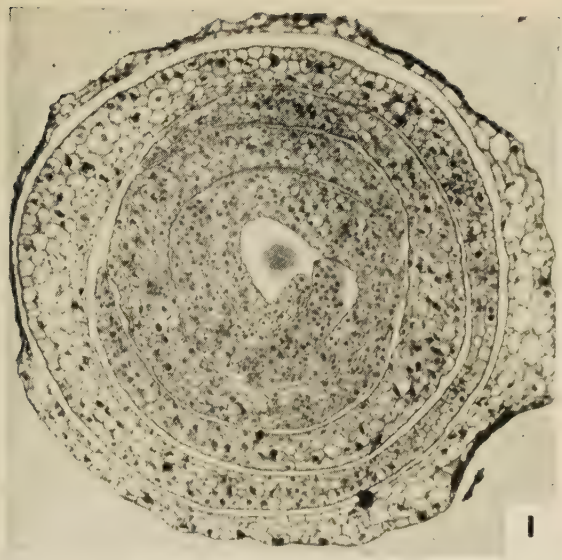
This saprophytic plant possesses somewhat reduced, brownish scale leaves which are arranged around the apex in an irregular spiral (Fig. 1), in contrast to the distichous arrangement in the autotrophic orchid *Orchis maculata* L. Since the apices in which the phyllotaxis was studied were approaching the flowering condition, however, it may be that the phyllotaxis deviated slightly from the normal vegetative condition.

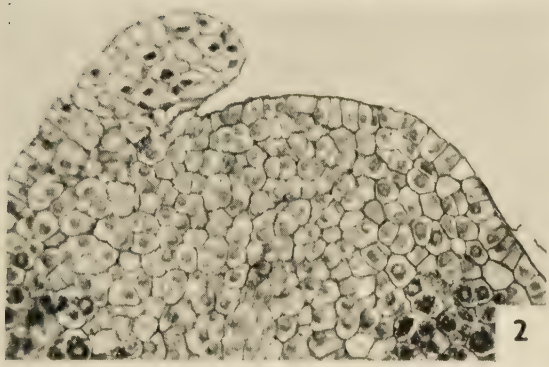
Apart from its saprophytic mode of life, the plant is of particular morphological interest due to the production of terminal root-buds, first reported by Prillieux (1856). Since the main shoot apices were usually found to be in the flowering condition, the apices of these root-buds have also been used in the study of apical anatomy.

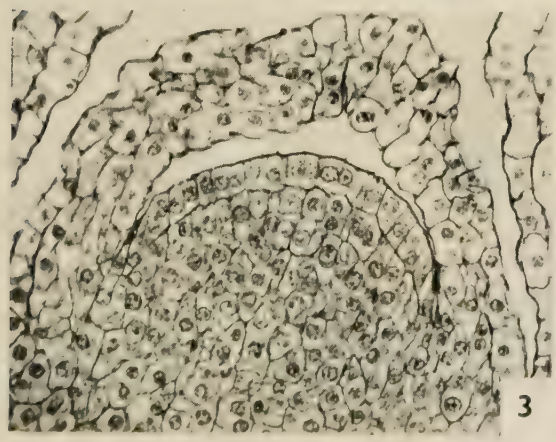
Apical Organization — The shoot apex is domed to a varying extent, becoming much more massive when approaching the flowering condition. The tunica is considered to be uniseriate, since occasionally only one clearly defined stratified layer may be present in the apex (Fig. 2). In general, however, vegetative apices possess two or three stratified layers (Figs. 3, 7-9). Periclinal divisions occur in the second and third layers, even in central positions. In flowering apices two stratified layers are present,

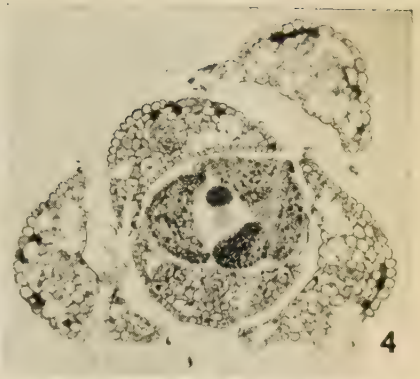
→

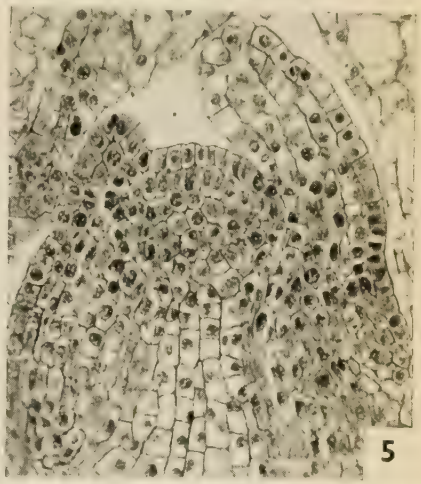
Figs. 1-6 — Fig. 1. *Neottia nidus-avis*, t.s. shoot apex. × 50. Fig. 2. *N. nidus-avis*, l.s. terminal root-bud with a young leaf primordium. The root tissues contain abundant starch (stained black). × 100. Fig. 3. *N. nidus-avis*, l.s. axillary bud. A periclinal division is present centrally in the second layer of cells. × 170. Fig. 4. *Cassytha filiformis*, t.s. shoot apex. The small size of the apex and the early vacuolation of the leaf primordia are evident. × 60. Fig. 5. *C. filiformis*, l.s. shoot apex. Two stratified layers of cells are present, and the cells are somewhat vacuolated. × 170. Fig. 6. *C. filiformis*, l.s. shoot apex, showing the prestelar tissue associated with a young leaf primordium on the right. × 170.











FIGS. 1-6.

but periclinal divisions may occur even in the tunica in the initiation of primordia.

In the lower regions of the corpus, divisions may occur in all planes, but a flank meristem in which divisions are predominantly anticlinal and periclinal is usually distinguishable from a central zone of cells in which divisions in all planes occur (Figs. 3, 9). Beneath the central zone the rib meristem is usually distinct. In flowering apices there is a much larger area of this central zone with irregular divisions. In general, flowering apices of *Orchis maculata* are very similar to those of *Neottia*, except that they are smaller and are composed of smaller cells.

The leaves of both *Neottia* and *Orchis maculata* consist of homogeneous mesophyll.

Axillary Buds — Organized buds may occur in the axils of P_6 and older leaves; their origin has not been traced. The tunica is uniseriate, but the second and third layers of the apical meristem are also stratified. Periclinal divisions, however, may occur centrally in the second layer (Figs. 3, 9). The rib and flank meristems are usually quite well marked.

Vascular Differentiation — Differentiation of prestelar tissue is acropetal; it was not present in P_1 until after its insertion on the axis. Sieve tubes are differentiated in the central vascular bundles of the leaves before there is any differentiation in the bundles of prestelar tissue in the axis. At a slightly lower level numerous empty, polygonal sieve tubes become differentiated on the outer side of the bundles of prestelar tissue in the axis; only very slightly later groups of polygonal cells become grouped on the inner side of the bundles, and commence lignification.

From a study of vascularization in the axis it was clear that phloem is the preponderant vascular tissue in the upper regions of the stem. Moreover, no xylem was observed in the scale leaves, whereas the sieve tubes, though not numerous, were quite distinct. This is in contrast to the condition in *Orchis maculata*, in the leaves of which prestelar tissue occurs in greater quantity, and in which both xylem and phloem are differentiated and become conspicuous.

Ergastic Substances — Starch is present in the scale leaves of *Neottia*, and also in the cells of the pith and inner cortex of the axis and rhizome. Starch grains are also present in the leaves of *Orchis maculata*, in addition to bundles of raphides.

CASSYTHA FILIFORMIS JACQUIN. (LAURACEAE)

This tropical plant, which may parasitize both herbaceous and woody plants (Chatin, 1892; Kienholz, 1926), resembles *Cuscuta* in habit, possessing a rather similar filiform stem which bears scale leaves.

Apical Organization — The apex is domed to a varying extent, and is rather small, only a small area being present above the leaves, which are arranged spirally (Fig. 4).

In all the specimens examined two stratified layers were present in the apices, but periclinal divisions occurred rather frequently in the second layer, usually in lateral positions and sometimes extending along almost the whole of one flank (Fig. 10). The tunica is therefore uniseriate. The cells of the second layer are sometimes considerably elongated in the proximal-distal plane. Except in the upper, stratified layer of the corpus, divisions occur in all planes; a central zone of cells upon which the files of rib meristem converge is evident (Figs. 5, 10). The

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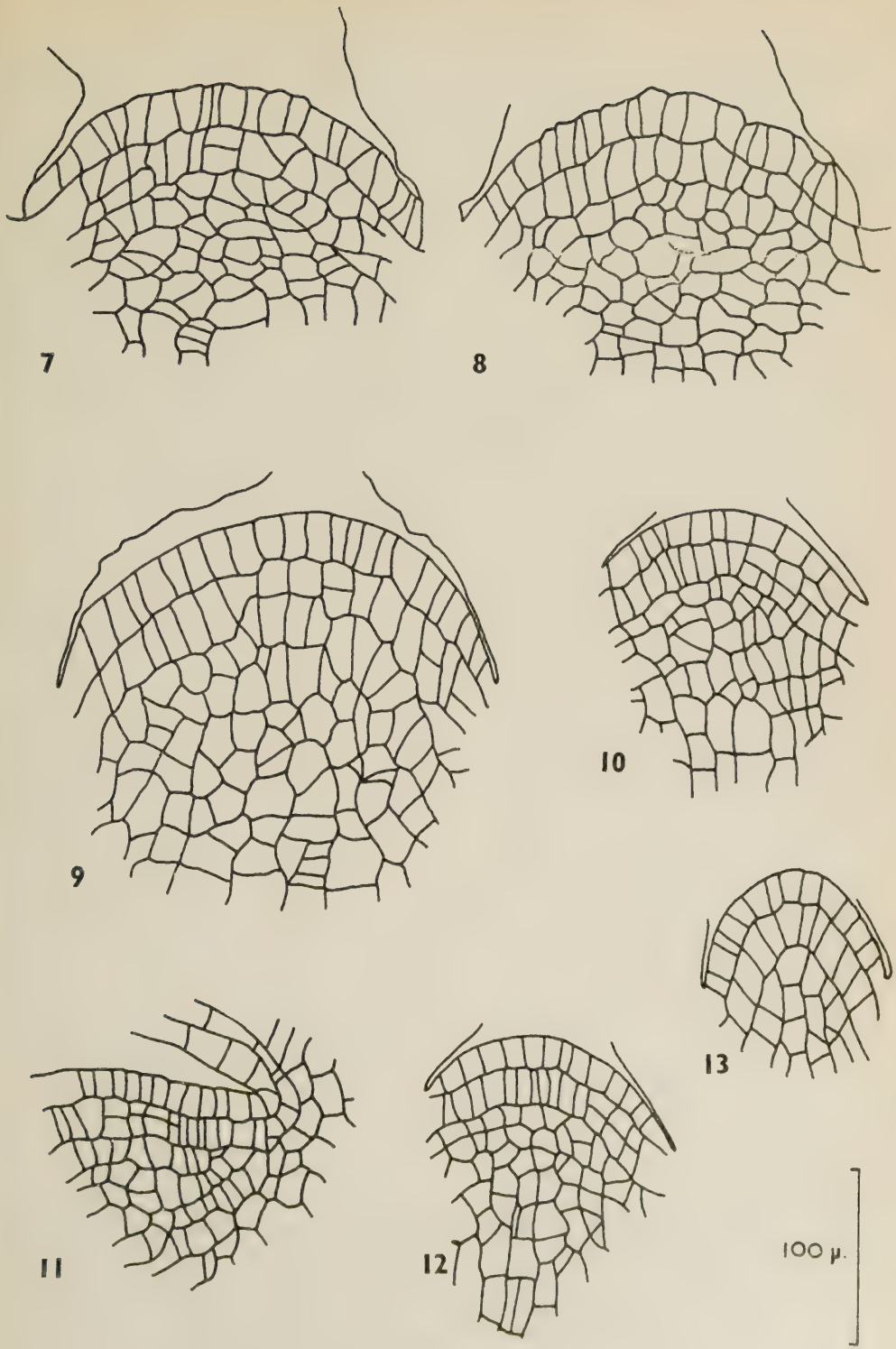
Figs. 7-13 — Figs. 7, 8. *Neottia nidus-avis*, l.s. root-bud. Fig. 7. median section; Fig. 8. section next but one. In Fig. 7 periclinal and oblique divisions are present in the second layer of cells. Fig. 9. *N. nidus-avis*, l.s. axillary bud, showing a periclinal division in the second layer of cells. Fig. 10. *Cassytha filiformis*, l.s. shoot apex; periclinal divisions are present in the second layer of cells along the right flank. Fig. 11. *C. filiformis*, l.s. shoot apex, showing an early stage in axillary bud formation. (Axillant leaf on the right.) Fig. 12. *C. filiformis*, l.s. axillary bud, showing two stratified layers of cells, with periclinal divisions along the right flank. Fig. 13. *Phoradendron flavescens*, l.s. axillary bud, showing two stratified layers of cells in the meristem, and distinct files of rib meristem. All ×255.



Figs. 7-13.

flank meristem was not well differentiated in the apices observed, perhaps because of the relatively small area of apex devoid of leaf primordia. Differentiation takes place rather rapidly below the apex, and even the cells of the apical meristem itself may be vacuolated to some extent (Fig. 5). Prestelar tissue was not observed above the level of the leaf primordia, but is very evident beneath even young primordia (Fig. 6).

The leaves, which arise by a periclinal division in the second layer, are rather reduced structures, and consist of homogeneous parenchyma in which very large cells occur in a sub-epidermal position; according to Mirande (1905) these are mucilage cells. Differentiation proceeds rapidly in the leaves, and the cells of quite young leaves are conspicuously vacuolated (Fig. 4). There is a single median vascular bundle.

Axillary Buds — Organized axillary buds may be present in the axils of P_5 upwards. The youngest stage of axillary bud formation observed (Fig. 11) resembles the detached meristems of the potato (Sussex, 1952). The histological organization of axillary buds is similar to that of the main shoot apex (Fig. 12), and two stratified layers are present, although periclinal divisions may occur even centrally in the second layer.

Vascular Differentiation — Differentiation of prestelar tissue in the leaves is acropetal; it is not present in the youngest leaves, but occurs below their insertion on the axis.

Basipetal sections of fairly young leaves show a central strand of prestelar tissue, in which sieve tubes first become differentiated. Immediately below this level the first xylem element becomes differentiated; on proceeding basipetally both xylem and phloem elements may increase in number, so that differentiation of vascular tissue in the leaves is presumably acropetal.

In older, vacuolated leaves extensive lignified tissue is present centrally; the elements are spirally and reticulately thickened. On proceeding basipetally this tissue decreases in extent, and prestelar cells appear in an abaxial position. At a lower level a normal vascular bundle with xylem and phloem is present; the lignified tissue appears to be continuous with the xylem. Since no lignified tissue of this sort is present in younger leaves, even those in which differentiated xylem and phloem are present, lignification must be a secondary phenomenon. Lignification presumably proceeds both in a basipetal and an acropetal direction, since basipetal sections show an increase and a subsequent decrease in the amount of this tissue. No mention of this extensive lignification is made in Mirande's (1905) account of the scale leaves of the stem; it may therefore be associated with seasonal factors or with particular host plants.

In the discrete vascular bundles of the young stem below the apex the sieve tubes increase in number and occupy a rather central position in the bundles of meristematic cells. Xylem is present only in the bundles associated with the older leaves; the other bundles consist entirely of phloem. At the lowest level sectioned a large cell with a prominent nucleus was becoming delineated in a central position in the phloem; according to Mirande (1905) these cells constitute the mother cells of the mucilage ducts, which eventually become conspicuous cavities.

Kienholz (1926) reports that a band of chlorophyll-bearing cells resembling palisade tissue is present in the stem. This was not observed in the young stem, which differs from the mature stem also in its possession of discrete vascular bundles.

Ergastic Substances — Starch is reported to occur in the endodermis of the stem (Mirande, 1905). The walls of the epidermal hairs which are present on the stem and scale leaves are frequently strongly thickened and lignified.

PHORADENDRON FLAVESCENS (PURSH.) NUTT. (LORANTHACEAE)

Specimens of this semi-parasite from Texas parasitizing both *Ulmus crassifolia* Nutt. and *Celtis occidentalis* L. have been examined. In general habit the plant resembles *Viscum album* L.

Apical Organization — The leaves are arranged in an opposite and decussate manner, and are large in proportion to the

shoot apex (Fig. 14). The latter varies in shape from a dome to a cone depending on the stage of the plastochrone.

Two stratified layers are always present in the apex, and although periclinal divisions do sometimes occur in the second layer they have not been observed in the central cells. The tunica is therefore biseriate. Planes of cell division in the corpus are variable; very clearly marked rib and flank meristems may be present directly beneath the tunica or the planes of division may be less regular. This appears to be related to the stage of the plastochrone. When the apex is of minimal area and the P_1's are large, division in the corpus is regular (Fig. 20); when leaf formation commences the flank meristem becomes obscured as a result of its activity in leaf inception, and the rib meristem is also less well marked (Figs. 21, 22). Further work is required to show whether this is generally true. The tunica cells are larger than those of the corpus, and possess large nuclei.

Leaf formation takes place by periclinal divisions in the second tunica layer and active division in the flank meristem (Figs. 15, 21). The leaves observed in the present study consist of uniform isodiametric mesophyll cells, but York (1909) states that the outer mesophyll cells of both leaf surfaces eventually become elongated and palisade-like. Epidermal hairs which may have lignified walls are present. There is a central vascular bundle and two smaller lateral bundles; anastomosis takes place between them.

The apex of *Viscum album*, which has not been studied in detail, is rather similar to that of *Phoradendron flavescens*, and is normally organized.

Axillary Buds — Organized axillary buds occur in the axils of the P_3's and older leaves; their origin at the shoot apex has not been traced. Cellular segmentation in axillary buds is similar to that of the main stem, and the flank and rib meristems may be very distinct (Fig. 13), although this is not always the case.

Vascular Differentiation — No prestelar tissue is present in the apex above the level of the youngest leaves, and all the existing vascular tissue observed in the stem was related to the leaves. Differentiation of prestelar tissue in the leaves is acropetal; it is not present in the youngest pair of leaves until below their insertion on the axis. Sieve tubes are present in the leaf traces before xylem elements, and differentiation is acropetal.

Ergastic Substances — Starch grains were not observed in the material examined, but York (1909) reports their occurrence in the inner cortex of the mature stem.

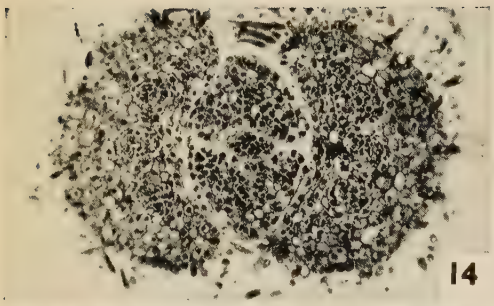
Both druses and stone cells with pitted walls occur in considerable numbers in the parenchymatous tissues of the leaves and axis. Cannon (1901) states that stone cells and crystals are characteristic of the old stems; in the present study, however, they were observed quite close to the apex. The walls of the unicellular hairs which occur in groups on the leaves and stem are thickly cuticularized and may also be lignified.

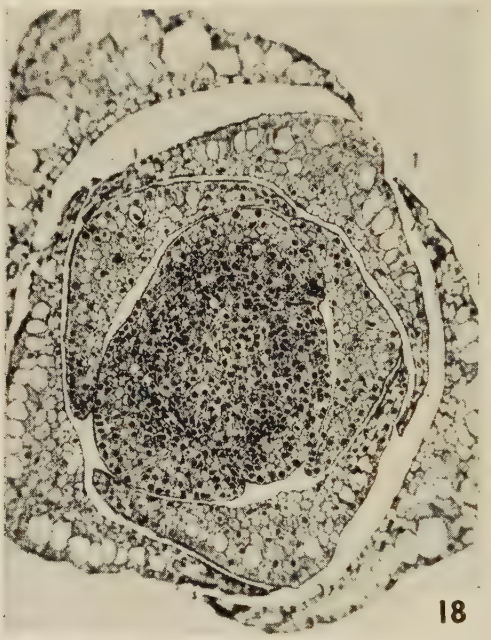
LORANTHUS GLOBIFERUS A. RICH. (LORANTHACEAE)

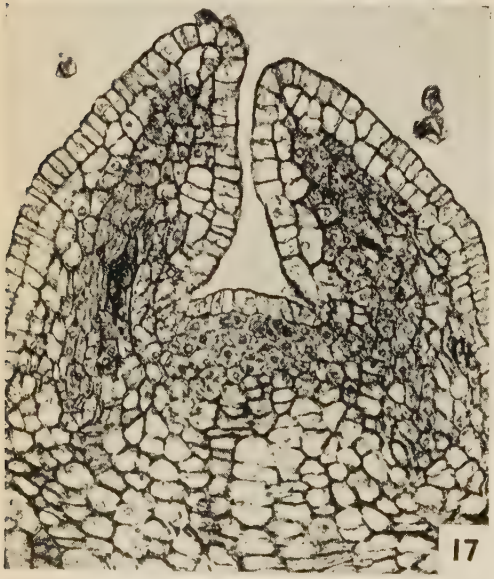
Shoot apices of this semi-parasite from northern Nigeria parasitic upon the shea-butter, *Butryospermum Parkii* Kotschy, were also examined, in less detail. Phyllotaxis is again opposite and decussate (Fig. 16).

Apical Organization — The apex is small in relation to the leaves, and may be almost flat or variously domed, according to the stage of the plastochrone. There may be only one layer of stratified cells in the apex; the tunica is therefore uniseriate. In some apices, however, up to three stratified layers may be present (Figs. 17, 23), and there is some indication that the number of stratified layers may be related to the stage of the plastochrone. The cells of the tunica, in contrast to those of the corpus, are rather vacuolate. The rib meristem is not very well marked, although there is a conspicuous pith, and there is little flank meristem. In the plane of leaf formation, divisions in the corpus seem to be more or less random, and these zones are not distinct.

The leaves consist of uniform isodiametric cells at the level sectioned. More







FIGS. 14-19.

mature leaves possess three vascular bundles, younger ones only one, as in *Phoradendron flavescens*.

Buds were not present in the apical regions; very small buds were present in the axils of older, excised leaves further down the axis.

VASCULAR DIFFERENTIATION — Differentiation of prestelar tissue is acropetal. Both xylem and phloem are quite well differentiated in the leaves. The structure and vascularization of the axis is similar to that of *Phoradendron*.

ERGASTIC SUBSTANCES — Starch was not observed in the tissues. Conspicuous groups of stone cells were observed in the pith of one specimen.

CUSCUTA EUROPAEA L. (CONVOLVULACEAE)

APICAL ORGANIZATION — The shoot apex of this parasitic plant is relatively large, and domed or parabolic in form. The scale leaves have their origin at some distance down the flanks of the apex, and are arranged in spiral phyllotactic sequence (Fig. 18).

A variable number of stratified layers of cells is present in the apex. In general, the tunica appears to be biseriate, although some periclinal divisions do occur in the second layer in both axillary buds and main shoot apices. At least two stratified layers are always present in the meristem. The third and fourth layers of cells are often also stratified (Figs. 19, 24). Mirande, who was one of the few authors to deal with apical regions of parasitic plants, also shows four stratified layers in the apex of *C. europaea* (Mirande, 1901, Plate V, Fig. 4).

Except in the upper, stratified layers, divisions occur in all planes in the corpus, but a flank-meristem zone where divisions are predominantly anticlinal and periclinal, and which is continuous with the prestelar tissue, can usually be distinguished from a central zone of small cells in which divisions are more irregular and oblique. The rib meristem consists of files of rather large, vacuolated cells. Prestelar tissue has not been observed in the apex above the level of the leaf primordia.

The inception of leaves takes place by a periclinal division in the second tunica layer; this agrees with the observations of Mirande (1901). The leaves consist of homogeneous mesophyll. A row of very large secretory cells, which may contain large nuclei but are usually almost devoid of cytoplasm, is present beneath the abaxial epidermis; according to Mirande (1901) these constitute latex-ducts. In the majority of the scale leaves around the apex a single median vascular bundle is present; this consists solely of a small number of polygonal sieve tubes (Fig. 18). In some of the older scale leaves small lateral bundles, of similar construction, may also be present.

AXILLARY BUDS — Organized axillary buds may be present in the axils of P_6 and older leaves. The tunica of the majority of axillary buds is biseriate (Fig. 25), and the third and even the fourth layers of cells are sometimes stratified. Histological organization is similar to that of the main shoot apex.

VASCULAR DIFFERENTIATION — Prestelar tissue was observed only in relation to the leaf positions, and differentiates acropetally into the leaf primordia. In the axis, differentiation of sieve tubes and of xylem elements appears to be approximately contemporaneous. Xylem is present in the vascular bundles right

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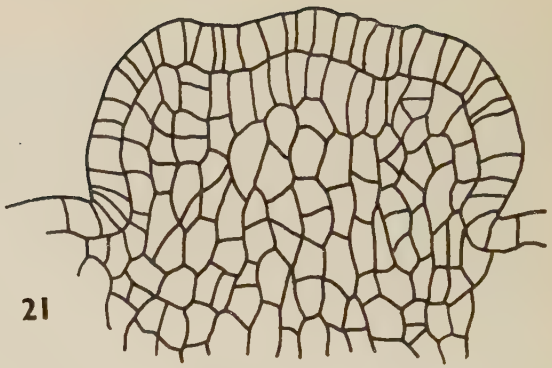
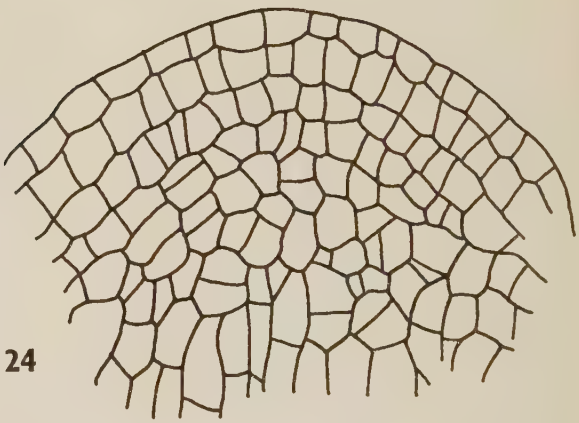
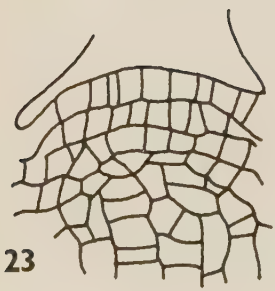
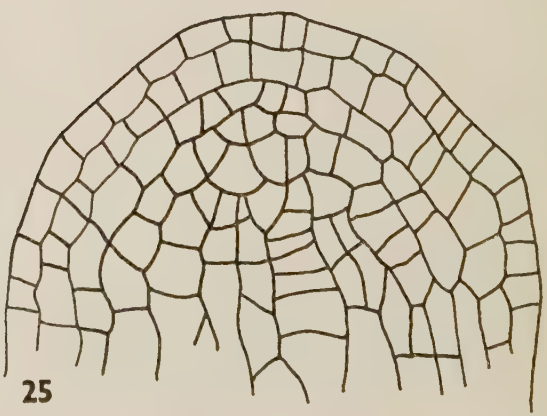
FIGS. 14-19 — Fig. 14. *Phoradendron flavescens*, t.s. shoot apex, showing the decussate phyllotaxis. Note the small size of the apex in relation to the leaves. Numerous epidermal hairs are present. × 60. Fig. 15. *P. flavescens*, l.s. shoot apex in the plane of foliar buttress formation. × 170. Fig. 16. *Loranthus globiferus*, t.s. shoot apex, showing the decussate arrangement of the leaves. Both sieve tubes and xylem elements can be distinguished in the leaf primordia. × 60. Fig. 17. *L. globiferus*, l.s. shoot apex in the plane of the P_1's. Three stratified layers of cells are present. × 170. Fig. 18. *Cuscuta europaea*, t.s. shoot apex showing the spiral phyllotaxis. Sieve tubes can be distinguished in the scale leaves; secretory cells are also evident. × 60. Fig. 19. *C. europaea*, l.s. shoot apex with a young leaf primordium. Three stratified layers are present in the meristem. × 170.









100 μ.

FIGS. 20-25.

up to the leaf axil, but is never present in the scale leaves. Indeed, as Mirande (1901) has already pointed out, the phloem is the predominant vascular tissue, and small bundles consisting of sieve tubes only are present in the axis.

Ergastic Substances — Starch grains are present in the scale leaves and in the pith of the axis, and have been reported in the cortical tissues of more mature parts of the stem (Chatin, 1892; Mirande, 1901).

CUSCUTA AMERICANA L.

This species is comparable with *C. europaea* to a considerable extent. The apex is more conical and more conspicuously stratified; it is also smaller and composed of smaller cells (compare Figs. 24, 26). The tunica is probably triseriate, since at least three stratified layers have always been observed in the material examined, even in axillary buds. Usually at least four layers are stratified. Apical organization is comparable with that of *C. europaea* in other respects (Figs. 26, 27).

The differentiation of the vascular tissue was not studied in detail, but appears to be generally comparable with differentiation in *C. europaea*. The scale leaves are reduced structures and are arranged spirally; like the leaves of *C. europaea* they possess large cells, or latex-ducts, in an abaxial position. A ring of these conspicuous latex-ducts is present also in the cortex of the axis; Mirande (1901) states that both latex-ducts and air-bearing tissues are particularly abundant in *C. americana*.

Starch is reported to be present in the stem (Mirande, 1901).

BALANOPHORA SP. (BALANOPHORACEAE)

Rhizome tips of a species of *Balanophora* from Malaya were examined. At the tips of the rhizome and its branches, an area of small, more densely-staining meristematic cells is present. This is Strigl's (1908) " Meristemkomplexe " of parasite tissue, and, as he observed, is rapidly transformed into tuber-parenchyma. No leaves are present on the rhizome. The tuber tissue consists of rather large-celled, homogeneous parenchyma; the epidermis is very irregular and the cells are often papillose. The walls of the epidermal cells are lignified.

Basipetal transverse sections of a rhizome tip show a number of groups of small, more densely-staining meristematic cells with large nuclei which stain with safranin. A few very large cells then appear in the centre of the groups of cells. Abundant small-celled tissue, presumably phloem, is present, and on proceeding basipetally xylem is differentiated towards the centre, next to the large cells which are centrally situated and possess very large nuclei and rather plasmolysed contents. The whole structure is surrounded by three to four layers of thin-walled vacuolate cells. Fusions between these groups of vascular tissue occur, and the number of strands therefore diminishes on proceeding basipetally.

There is much controversy in the literature regarding the interpretation of these strands of conducting tissue. Van Tieghem (1907) considers that they are the true steles of the parasite, while Heinricher (1908) and Strigl (1908) claim that Van Tieghem's steles are in fact branches which grow into the tuber from the roots of the host plant, the large central cells being the thallus of the parasite. The limited observations of the present study cannot resolve this controversy.

Buds arise endogenously in the lobes of the rhizome and give rise to the inflorescence axis, which bears scale leaves (Heinricher, 1908; Strigl, 1908; Van Tieghem, 1907). The organization of these apices is not described in the literature, and no

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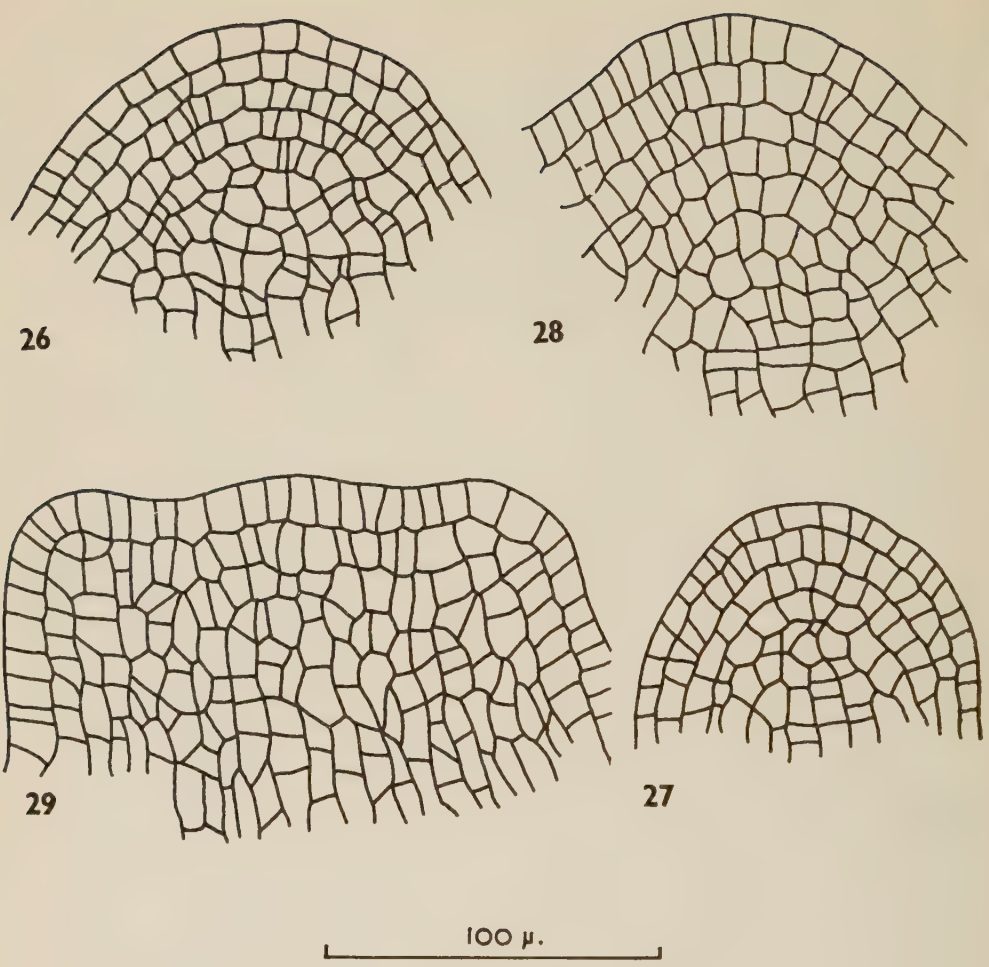
Figs. 20-25 - Figs. 20-22. *Phoradendron flavescens*, l.s. shoot apex in the planes of the P_1's, foliar buttress formation, and leaf formation respectively; rib and flank meristems are very well marked in Fig. 20. Fig. 23. *Loranthus globiferus*, l.s. shoot apex, showing three stratified layers of cells. Fig. 24. *Cuscuta europaea*, l.s. shoot apex, showing four stratified layers of cells. Fig. 25. *C. europaea*, l.s. axillary bud; the rib meristem is very well marked. All Figs. × 255.



FIGS. 26-29 — Fig. 26. *Cuscuta americana*, l.s. shoot apex, showing five stratified layers of cells. Fig. 27. *C. americana*, l.s. axillary bud. Four stratified layers of cells are present, and the central zone and rib meristem are distinct. Fig. 28. *Lathraea clandestina*, l.s. shoot apex. Five stratified layers of cells are present. Fig. 29. *L. clandestina*, l.s. axillary bud in the plane of foliar buttress formation. Two stratified layers of cells are present, and files of prestelar tissue occur beneath the leaf primordia. All Figs. × 440.

organized meristematic tissue was observed in the present study. The growing points of the tuber are thought to occur in the meristematic complex of parasite tissues situated upon the tips of the " host root branches " (Strigl, 1908).

The scale leaves borne on the inflorescence axis are said to possess several vascular bundles, and the epidermis may be lignified (Van Tieghem, 1907).

No starch is present in the rhizome or inflorescence axis, but a resinous substance — balanophorin — occurs. (Metcalfe & Chalk, 1950; Van Tieghem, 1907.)

LATHRAEA CLANDESTINA L. (OROBANCHACEAE)

A few specimens of this parasite of poplar and willow roots were examined. In addition a few apices of *Orobanche hederae* Duby and *Aeginetia indica* L. were sectioned, but they were found to be in the flowering condition.

Insufficient material was examined to reach a reliable estimate of the number of tunica layers in the species, but in the apices observed the tunica was biseriate and up to four stratified layers were present (Fig. 28). A small central zone of cells in which divisions are irregular and oblique is present, beneath which files of rather vacuolate rib meristem cells occur. The flank meristem is not well marked.

The histological organization of axillary buds is similar to that of the main apex, except that fewer stratified layers may be present (Fig. 29). Leaves are initiated by a periclinal division in the second layer of cells, and prestelar tissue is present beneath them.

The scale leaves consist of homogeneous mesophyll and possess central cavities into which project numerous glands, which function as hydathodes (Metcalfe & Chalk, 1950).

A ring of vascular tissue is present in the axis, with groups of xylem centrally. Both xylem and phloem are differentiated in the scale leaves. In *Orobanche hederae* the axial bundles form an almost complete ring in the flowering axis at the level sectioned, and in *Aeginetia indica* discrete vascular bundles were present in the material examined.

Starch grains are present in considerable abundance in the parenchymatous tissues of the axis and scale leaves. Starch was observed also in the pith and cortex of the flowering axis of *Aeginetia indica.*

Discussion

The foregoing brief anatomical descriptions of the shoot apices of a number of saprophytic and parasitic flowering plants show that anomalous nutrition of this kind does not of itself cause abnormality in the organization of apical tissues. The species studied all exhibited the normal apical structure of monocotyledons and dicotyledons, with the exception of the rhizome of *Balanophora.* The rhizome apex of this species, which shows the greatest morphological anomaly of those studied, did not possess the organization of a normal shoot apex, but it remains possible that the endogenous inflorescence apex, which does not seem to have been described, is normally organized. The apex of *Arceuthobium pusillum* Peck, illustrated diagrammatically but not described by Thoday & Johnson (1930b, Fig. 3), appears also to be normal, notwithstanding the reduction of the vegetative phase of the plant to a number of endophytic strands running within the host tissues (Thoday & Johnson, 1930a). An examination of the apical anatomy of insectivorous plants would be an interesting complementary study.

It is possible that the effect of nutrition in morphogenesis may be mainly on the rates of metabolic processes, within the fundamental reaction system characteristic of the species. Wetmore (1950, 1954) has studied the effect of varying concentrations of essential nutrients and of the addition of various growth-promoting substances upon the growth *in vitro* of shoot apices of a number of angiosperms and pteridophytes; he found that the rate of growth was affected rather than the developmental pattern. From such evidence Wetmore & Wardlaw (1951) conclude that the developmental pattern of the species is sufficiently stable to withstand considerable variation in nutrient supply. The mode of nutrition of parasites and saprophytes, although "irregular", is nevertheless evidently efficient, after the initial hazards of finding a host have been overcome, and is adequate to maintain growth; the existence of normal apical organization in these plants is therefore not entirely an unexpected finding. Furthermore, the path of nutrients to the apex in these shoots must still be acropetal, and indeed distribution of nutrients within the apex may well be similar to that suggested by Wardlaw (1952) for the apex of the fern *Dryopteris aristata.*

The prevalence of deposits of starch among the species studied is noteworthy, and indicates that carbohydrate nutrition was not limiting. Starch was observed, or had been previously reported to occur, in the tissues of all the species examined except *Loranthus globiferus* and *Balanophora.* Juliano (1935) comments upon the remarkable storage of starch in *Aeginetia indica.* The almost universal occurrence of mechanical tissue of various kinds

in these plants is also very striking. Of the species studied, numerous stone cells were observed in *Loranthus* and *Phoradendron*, lignification of the epidermal cells or of epidermal hairs in *Balanophora*, *Cassytha* and *Phoradendron*, lignified tissue in the leaves of *Cassytha*, and occasional lignified cortical cells in *Neottia*. Metcalfe & Chalk (1950) record the presence of stone cells in other parasitic species of the Loranthaceae, Balanophoraceae and Santalaceae; mechanical tissue in species of the Orobanchaceae, Santalaceae and Loranthaceae; and thick-walled, pitted or lignified parenchymatous cells in species of the Loranthaceae, Orobanchaceae and Balanophoraceae. No mechanical tissue was observed in the species of *Cuscuta* examined in the present investigation, but Mirande (1901) records the occurrence of sclerides in the cortex and round the haustoria in some species, and of sclerified pith in others. While it is realized that mechanical tissues are also commonly found in autotrophic plants, their widespread occurrence in saprophytic and parasitic species is very remarkable. This point is of particular interest in view of the work of Burkholder & McVeigh (1940) on the effect of nitrogen supply upon the anatomy of maize plants. They found that plants suffering from nitrogen deficiency exhibited xeromorphic characters and were stiff and woody due to the formation of thick cell walls, sclerenchymatous tissue, fibres and so on. They point out that when a considerable amount of available nitrogen is present, carbohydrates are used up in the synthesis of proteins and are not available for the thickening and lignification of cell-walls. The prevalence of mechanical tissues among parasitic plants is therefore further evidence that the supply of carbohydrates is adequate, and suggests that nitrogen may be limiting. Furthermore, Burkholder & McVeigh (1940) also observed that there was a reduction in the size and number of cortical, pith and vascular elements in the stems of plants deficient in nitrogen, and that the apical regions developed in direct proportion to the amount of nitrogen supplied. The small size of the apex relative to the leaves in such species as *Phoradendron flavescens*, *Loranthus globiferus*, and even *Cassytha filiformis* has been commented upon above. It is possible that nitrogen and other mineral nutrients may be more limiting at certain seasons of the year, especially in plants which parasitize deciduous trees.

All the completely heterotrophic species studied possess reduced scale leaves. These leaves are not smaller at their inception than those of autotrophic plants, but evidently do not undergo any considerable further development during ontogeny. They consist of homogeneous mesophyll and do not function to any extent as photosynthetic organs. Another feature common to these species is the preponderance of the phloem tissue over the xylem. Both *Cassytha filiformis* and *Cuscuta* spp. possess bundles in the young axis consisting solely of phloem, and in *Neottia* phloem is present considerably in excess of the xylem. Metcalfe & Chalk (1950) also report that phloem is more fully developed than xylem in the stems of *Lathraea squamaria* and *Orobanche hederae*, and Juliano (1935) states that in *Aeginetia indica* also the xylem is reduced and the phloem well developed. In contrast to this, Thoday & Johnson (1930a) state that no phloem is present in *Arceuthobium pusillum*, either in the endophytic system or in the aerial shoot; it is, however, illustrated in the stem of *Arceuthobium oxycedri* Marsch. Bieb. (Metcalfe & Chalk, 1950, Fig. 287 B). The conclusion of Jacobs (1952) that auxin is the limiting factor in xylem differentiation is of some interest, especially in view of possible correlation between growth hormone content of the shoot apex and the nitrogen supply to the plant (Avery, Burkholder, & Creighton, 1937).

The embryology of these plants with anomalous nutrition is of considerable interest, and here greater abnormality exists than in the shoot apices. Apomixis is a feature of the Balanophoraceae (Johansen, 1950), and reduced embryos which are not organized into plumule, radicle and cotyledons are found in the Balanophoraceae, Rafflesiaceae, Gentianaceae, Pirolaceae, Orobanchaceae, Burmanniaceae and Orchidaceae (Maheshwari, 1950). Maheshwari considers that such reduced embryos are

associated to some extent with a parasitic or saprophytic habit of growth, although they do occur also in a few autotrophic species. The embryo of *Cuscuta reflexa* is also devoid of cotyledons (Johri & Tiagi, 1952), as is that of *Arceuthobium pusillum* (Thoday & Johnson 1930b). In view of Wardlaw's (1953b, 1954) finding that the organization of the endogenous root- and shoot-buds of the fern *Ophioglossum vulgatum* differs from that reported for the embryo, particularly in the sequence of organogenesis, it is of some interest to note that while the embryo of *Aeginetia indica* shows no differentiation into plumule, radicle and cotyledons (Kusano, 1908), the endogenous shoot apex is apparently normally organized (Juliano, 1935, Figs. 87, 88). Endogenous shoot buds occur also in *Christisonia* (Worsdell, 1895) and *Balanophora* (Heinricher, 1908; Strigl, 1908; Van Tieghem, 1907), but as far as the writer is aware, their organization has not been described. Discussing the case of *Ophioglossum*, Wardlaw (1953b, 1954) points out that the nutritional environment of the embryo and the endogenous bud rudiment is very different. In the case of these parasitic and saprophytic plants therefore it may be that the nutritional status of the adult shoot apex is not sufficiently limiting to cause any deviation from normal shoot morphology, whereas the embryo may develop under less favourable conditions.

It is probable that the methods of tissue culture will prove the most promising technique for the study of the effect of nutrition upon plant morphogenesis, both in autotrophic and heterotrophic plants. Seedlings of *Cuscuta* have already been successfully grown *in vitro* (Loo, 1946; Molliard, 1908), and Gaertner (1950) has grown seedlings of *Cuscuta* and its host together in sterile culture. Loo (1946) has also cultured excised stem tips of *Cuscuta campestris*; no roots or leaves developed, although floral organs were formed. Future work upon heterotrophic plants may therefore turn from the investigation of problems of germination to a study of precise nutrient requirements by means of tissue culture techniques. The culture of excised apices of such plants, already successfully carried out with *Cuscuta* by Loo (1946), might provide interesting information, although it seems probable that their *in vitro* requirements will be similar to those of the apices of autotrophic plants. Such investigations should be preceded by an exhaustive study of the apical anatomy of these plants, which is apparently a neglected field.

Summary

An anatomical investigation of the shoot apices of some angiosperms with anomalous modes of nutrition has been carried out with a view to determining whether their abnormal nutrition has an effect upon apical organization.

The apical anatomy of one saprophytic and seven parasitic species of flowering plants is briefly described, and it is concluded that there is no anomaly of shoot apical organization associated with irregular nutrition. Those species studied which were wholly heterotrophic possessed reduced scale leaves, and phloem was the preponderant vascular tissue. The prevalence of starch and mechanical tissues in these species indicates that carbohydrate supply is adequate and suggests that nitrogen may be limiting.

It is suggested that further studies of heterotrophic plants by the methods of tissue culture might be fruitful.

I wish to express my gratitude to Professor C. W. Wardlaw for suggesting the problem, and for much valuable advice and encouragement during the course of the investigation. My sincere thanks are also due to Mr. E. Ashby and Mr. G. Barker for the photographic illustrations, and to the following people to whom I am indebted for permission to collect material or for collecting and transmitting material of exotic parasitic species: Sir William Wright Smith, Regius Keeper of the Royal Botanic Garden, Edinburgh; Dr. J. A. Macdonald, Interim Keeper of the University Botanic Gardens, St. Andrews; Professor R. E. Holttum; Professor C. Heimsch and Professor R. H. Wetmore; Dr. L. Hawker; and Miss E. Harrison.

This work was carried out during the tenure of a Berry Scholarship awarded by the University of St. Andrews, for which grateful acknowledgement is made.

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SPOROGENESIS AND THE FEMALE GAMETOPHYTE OF *PHORMIUM TENAX*

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Introduction

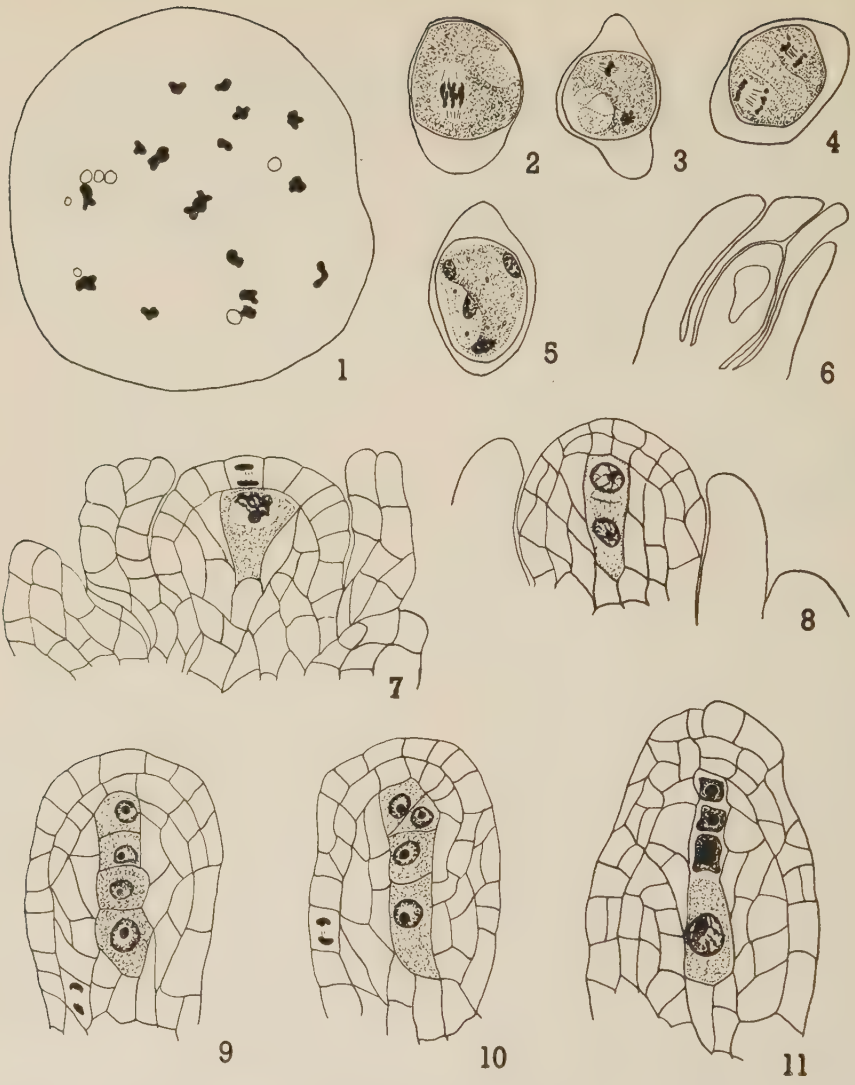
In his treatment of the Liliaceae in Engler & Prantl's Pflanzenfamilien, Krause (1930) placed the genus *Phormium* in the tribe Hemerocallideae, together with *Hosta*, *Hemerocallis*, *Hesperocallis*, *Leucocrinum*, and *Blandfordia*. Most taxonomists have treated *Phormium* in this manner, but Hutchinson in 1934 constituted the family Agavaceae, in which he united the large, woody, and often xerophytic genera from the liliaceous subfamily Dracaenoideae with the Agavoideae of the family Amaryllidaceae and the genus *Phormium*. He subdivided the family into six tribes: Yucceae, Dracaeneae, Phormieae, Nolineae, Agaveae, and Polyantheae.

A survey of embryological features of four genera in the Hemerocallideae (Cave, 1948) shows a close relationship of *Hosta* and *Hesperocallis* with each other and with the *Yucca-Agave* group, but not with *Leucocrinum* or *Hemerocallis*. Nor do the two latter seem to be closely related to each other.

From the available information Wunderlich (1950) made a comparative study of the embryology and carpel, stamen, and leaf structure of the Agavaceae. The evidence supports the placement of *Yucca* and *Agave* in the same family, but indicates that Hutchinson's tribes do not show the most natural relationships among the genera. Wunderlich divides the family into four groups as shown in Table 1, omitting *Phormium* and *Doryanthes*, the former because of lack of information. Joshi & Pantulu (1941) exclude the latter from the Agaveae and place it in a tribe of its own, the Doryantheae. Wunderlich raises the question as to whether these four heterogeneous groups should be united into one family and whether *Phormium* and *Doryanthes* should be excluded.

Although *Phormium tenax* is an important economic plant, there has been little purely botanical work done on it[1]. The

1. In correspondence in 1948 Mr. M. E. Roberts of the *Phormium* Research Station at Foxton, New Zealand, stated that he was making an embryological study of *Phormium tenax*, and had found that the embryo sac developed according to the normal type. The present author has been unable to locate any publication of the work and has been unsuccessful in subsequent efforts to correspond with Mr. Roberts.



FIGS. 1-11 — Fig. 1. Diakinesis in PMC showing 16 bivalents and 7 nucleoli. × 1180. Fig. 2. Metaphase of heterotypic division in PMC. Fig. 3. Metaphase of homeotypic division. Fig. 4. Anaphase of homeotypic division. Fig. 5. Four nuclei before delimitation of microspores. All Figs. × 540. Fig. 6. Four-nucleate embryo sacsurrounded by nucellus and integuments. × 125. Fig. 7. Prophase of meiosis in EMC. Note periclinal division in cell of nucellar epidermis. Fig. 8. Telophase of heterotypic division. Fig. 9. Megaspores. Fig. 10. Same. Fig. 11. Uni-nucleate gametophyte. All Figs. × 540.

present paper is a description of its sporogenesis and megagametophyte development, prepared in the hope of obtaining evidence to show whether its affinities are with the Agavaceae or with the Hemerocallideae, or with neither.

Material and Methods

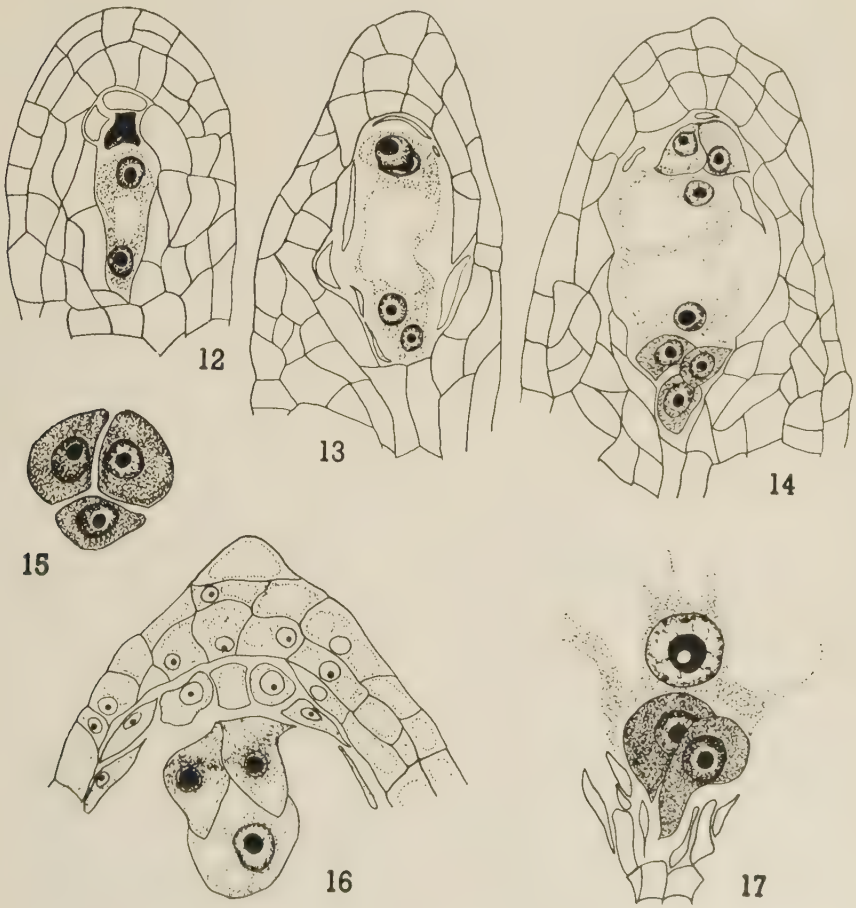
Material was obtained from plants cultivated in 1948 at the Botanical Garden of the University of California in Berkeley. Very young buds were fixed in 3 parts

absolute alcohol to 1 part glacial acetic acid, and studies of microsporogenesis and chromosome number were made from aceto-carmine smears. Slides were made permanent by the vapor exchange method. Material used for the study of megasporogenesis and the female gametophyte was fixed in CRAF, dehydrated in normal butyl alcohol, embedded in paraffin, cut at 10 and 20 microns, stained in Heidenhain's iron alum haematoxylon, and mounted in balsam or piccolite.

Microsporogenesis

There are 16 bivalents at diakinesis (Fig. 1). Numerous nucleoli may be seen. Some of them are associated with bivalents, whereas others lie free. The squashing in making the preparation may have broken them away from their particular chromosome pairs.

At metaphase of the first meiotic division the protoplast of the pollen mother cell already shows a polarization, the



FIGS. 12-17 — Fig. 12. Two-nucleate gametophyte. Note remains of megaspores and periclinal divisions of nucellar epidermis. Fig. 13. Four-nucleate gametophyte. Fig. 14. Eight-nucleate, 7-celled gametophyte, one cell of the egg apparatus not shown. Fig. 15. T.s. antipodal cells of mature gametophyte. Fig. 16. Micropylar end of mature gametophyte showing egg, synergids, and nucellar cap. Fig. 17. L.s. chalazal end of mature gametophyte showing two of the three antipodal cells, the primary endosperm nucleus, and disintegrating nucellar cells. All Figs. × 540.

nucleus lying at one side of a cup-shaped protoplast (Fig. 2). The second division takes place with the nuclei at opposite sides of the cup-shaped structure (Figs. 3, 4). At the end of telophase II, two of the spore nuclei lie near the rim of the cup and two below (Fig. 5). Delimitation of the microspores is simultaneous.

Megasporogenesis and the Female Gametophyte

It has been reported (Cave, 1953) that a primary parietal cell was formed by the female archesporial cell in the nucellus of the ovule. Early stages in meiosis show cells between the embryo sac mother cell and the nucellar epidermis (Fig. 8). If younger stages are examined it will be seen that these cells arise, not from the archesporial cell, but from the nucellar epidermis (Fig. 7), and *Phormium* must, therefore, be listed among those plants without a parietal or cover cell.

Meiosis results in a linear row of megaspores, although the two micropylar spores sometimes lie at an oblique angle to one another (Figs. 9, 10). The chalazal spore enlarges and gives rise to an 8-nucleate gametophyte which is organized in the usual manner (Figs. 11-14).

During the growth of the female gametophyte the cells of the nucellar epidermis and those below divide periclinally and anticlinally to form a tissue several rows thick at the micropylar end. Maximum thickness of the nucellar cap is attained at about the 2-nucleate stage of the embryo sac. After that the growth of the gametophyte causes the nucellar layers next to it to disintegrate (Figs. 12-14, 16). The mature sac just before fertilization has about three layers of large vacuolate nucellar cells forming a cap at the micropyle and shows the remains of nucellar cells adjacent to the gametophyte.

Both inner and outer integuments are present. The former is two cells thick at the sides of the sac, increasing to six to eight at the top where it forms the micropyle (Fig. 6).

The cytoplasm of the synergids of the mature gametophyte stains darkly and a faint filiform apparatus is discernible. The egg has a large vacuole (Fig. 16). The polar nuclei fuse at the center of the sac and then migrate toward the antipodal cells, suggesting that *Phormium* may have the Helobial type of endosperm development.

In cross-section the large antipodal cells with dense cytoplasm are wedge-shaped (Fig. 15), but in longitudinal section they are drawn out into beak-like structures which extend into the chalazal end of the nucellus (Fig. 17). The walls of the chalazal cells do not thicken to form a hypostase, but rather seem to show evidence of disintegration as do the cells at the sides and top of the growing gametophyte.

Discussion

Genera of the two groups to which *Phormium* has been assigned are listed in Table 1, together with certain of their embryological and cytological characteristics and geographic distribution. For ease of comparison *Phormium* is placed between the two groups. The upper group comprises those genera of the Hemerocallideae of the family Liliaceae on which embryological work has been done. The lower contains such genera of the Agavaceae arranged according to the classification of Wunderlich.

On the basis of delimitation of microspores *Phormium* does not show affinities with any member of the Hemerocallideae. Although *Hemerocallis* has been reported as having simultaneous wall formation in the delimitation of microspores (Cave, 1948), it has been shown by Yamaha (1926) that between the nuclei at the end of the first meiotic division a cell plate is begun, but the spore nuclei are not completely separated from each other until the close of the second meiotic division. Schnarf (1931) considers *Hemerocallis* fundamentally as having successive microspore delimitation and it should probably be listed thus.

In comparing other embryological characteristics of *Phormium* with those of the genera of the Hemerocallideae, it will be seen that none has the same combination

TABLE 1 — CYTOLOGICAL AND EMBRYOLOGICAL CHARACTERISTICS AND GEOGRAPHICAL DISTRIBUTION OF THE HEMEROCALLIDEAE AND AGAVACEAE[1]

Genus	Basic Chrom. No.	Delim. of Micro.	Cover Cell	Periclinal Div. in Nucellar Epidermis	Nucellar Cap	Hypostase	Embryo Sac Shape	Antipodals	Geog. Distrib.
Hemerocallideae									
Hemerocallis	11	succ. ±	0	0	0	0	ovate	large	Old World
Leucocrinum	14	succ.	+	+	0	±	ovate	large, binuc.	New World
Hesperocallis	24	succ.	+	0	0	+	broad, chalazal constriction	small	New World
Hosta	30	succ.	+	0	0	+	broad, chalazal constriction	large	Old World
Phormium	16	sim.	0	+	+	0	ovate	large	N.Z.
Agavaceae									
Yucca	30	succ.	+	0	0	+	broad, chalazal constriction	small	New World
Beschorneria	30	—	+	0	0	+	broad, chalazal constriction	small	New World
Furcraea	30	succ.	+	0	0	+	broad, chalazal constriction	small	New World
Agave	30	succ.	+	0	0	+	broad, chalazal constriction	small	New World
Polyanthes	30	succ.	+	0	0	+	broad, chalazal constriction	med., binuc.	New World
Nolina	18, 19	succ.	0	—	—	—	—	small, degen.	New World
Dasylirion	19	succ.	0	0	0	+	broad, chalazal constriction	small, degen.	New World
Dracaena	19	succ.	0	+	—	+	narrow	small	Old World
Sansevieria	20, 21	succ.	0	+	—	—	narrow	small	Old World
Cordyline	19	succ.	+	+	—	—	broad	large	Old World[2]
Doryanthes	18, 24	sim.	+	+	+	+	broad, chalazal constriction	small, multipl.	Australia

1. Data assembled from accounts in the literature and personal observations. A comprehensive bibliography concerning embryological studies on the genera listed in the table is to be found in Wunderlich (1950).

2. Except for the South American *Cordyline dracaenoides*.

of characters as is found in *Phormium*. *Hemerocallis* does not form a primary parietal cell and has an ovate mature sac with no hypostase, but the nucellar epidermis does not show periclinal divisions which produce a nucellar cap at the micropyle. *Leucocrinum* shows some similarity with *Phormium* in the large antipodals and periclinal divisions of the nucellar epidermis, but the antipodals are binucleate and there is no nucellar cap formed. *Hosta* and *Hesperocallis* differ from *Phormium* in all the embryological characteristics listed in the table. The latter genus would therefore seem to show no close affinities with the other members of the tribe Hemerocallideae.

Among the Agavaceae *Doryanthes* is the only genus having simultaneous delimitation of microspores. It is also the only one with periclinal divisions in the nucellar epidermis and a nucellar cap over the embryo sac. Furthermore, its geographic distribution is closer to that of *Phormium* than is that of any other genus of the family. These comparisons at first suggest that other characters might show similarities between the two genera. However, the broad upper part of the embryo sac in *Doryanthes* and its chalazal constriction, limited by thick walled cells of the hypostase, are very different from the ovate sac and the thin walled cells of the chalaza in *Phormium*. The very large antipodal cells of the latter are not seen in any other genus of the Agavaceae except *Cordyline*. According to Newman (1928, 1929) the nucellar cap originates in *Doryanthes excelsa* from a primary parietal cell which has been cut off by the archesporial cell. From his figures and from observations of the present author on *D. palmeri* there is a considerable tissue over the EMC before meiosis takes place. Newman's figures do not show periclinal division of the nucellar epidermis at the sides of the sac, but in *D. palmeri* such divisions are frequent. The possibility that the nucellar cap in *Doryanthes* may have arisen from the nucellar epidermis as is the case in *Phormium* cannot be excluded.

The chromosome number of *Phormium* offers no clue as to its relationships, other than to point out the gulf between it and the members of the *Yucca-Agave* group.

Schnarf (1931) considers the type of microspore delimitation one of the most important systematic characters in embryology. If this is so, *Doryanthes* is the only genus which conceivably might show close affinities with *Phormium*. In view of the distinct differences in the mature gametophytes of the two genera, the difference in chromosome number, and the possible difference in origin of the nucellar cap, it is questionable whether the relationship is a close one. Joshi & Pantulu suggest that *Doryanthes* should be placed in a tribe of its own. Hutchinson considers *Phormium* as the only genus in its tribe. As stated by Wunderlich, the question as to whether all the heterogeneous groups in the Agavaceae should remain united into one family still remains open. At present the evidence seems to show that both *Doryanthes* and *Phormium* should be excluded from the family. Comparative analytical studies in other fields would be most desirable in elucidating this problem, for knowledge of the true affinities of the two genera requires much more information about monocotyledonous families than is now available.

Summary

Embryological studies on *Phormium tenax* indicate that the genus *Phormium* is not closely allied to the genera of the tribe Hemerocallideae of the Liliaceae, where it has been placed by Krause in Engler & Prantl's Pflanzenfamilien. Hutchinson ascribes *Phormium* to the Agavaceae, but the above studies further show that it has no close affinities with the genera of this family, with the possible exception of *Doryanthes*. Both *Phormium* and *Doryanthes* have simultaneous delimitation of microspores. This character, important taxonomically suggests a relationship between these two genera although not a close one, when other embryological characters are considered. It is probable that neither of these genera should be included in the Agavaceae.

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MORPHOGENESIS IN *SOLANUM TUBEROSUM* L.: APICAL STRUCTURE AND DEVELOPMENTAL PATTERN OF THE JUVENILE SHOOT

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Introduction

In the shoot apex of angiosperms there may be little evident histological distinction between cells which comprise the apical meristem and those commencing to differentiate on the flanks of the apical mound. However, the potentialities for further development of meristematic and differentiating cells differ considerably, and in order to understand more clearly the role of the apical meristem in morphogenesis of the shoot it is desirable to obtain precise information concerning the growth of its component cells, and its vertical and lateral extent at the shoot apex. Wardlaw (1943a, b) was able to delimit the apical meristem in the shoot of various fern species by the distinctive appearance of the superficial layer of prismatic cells, but the apical meristem in the shoot of an angiosperm has not yet been defined with the same accuracy.

This investigation was undertaken to delimit the apical meristem in the juvenile shoot of the potato, and to describe also histological changes which occur during differentiation of tissues in the stem, and the formation of lateral organs. These findings will be used subsequently as a background against which to interpret experimental studies of morpho-

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genesis, some of which have previously been reported briefly (Sussex, 1954).

Material and Methods

The terminal regions of the potato shoot have been investigated with special reference to the varieties Kerr's Pink, Arran Banner, Arran Peak, Majestic, Stormont Dawn and Ulster Chieftain. These varieties cover a range of mature morphological form and were found to be suitable for experimental treatment. *SS* or *A* certified seed potatoes were used whenever possible as a source of material to ensure varietal constancy and freedom from virus infection.

Detailed anatomical examination has been confined to shoots which had only recently begun to grow from the tuber. To obtain such juvenile shoots, tubers were stored in darkness or in diffuse light at room temperature until axillary buds had grown to a length of about 5 mm. " Eyes " which contained such growing shoots were then cut from the tubers using a 15 mm diameter cork borer, and the plugs of tuber tissue were trimmed basally to a length of 15-20 mm. Only one growing bud was allowed to remain on each tuber plug, any additional buds being trimmed off. The tuber plugs were kept in covered glass dishes on filter paper moistened with Knop's solution (Gautheret, 1942), and the dishes were placed in a dark incubator maintained at 25°C. After about 7 days, shoots 15-20 mm in length, with a pale yellow stem, bearing numerous adventitious roots, and leaves 2-4 mm long and yellow or coloured according to the variety, were fixed for anatomical study (Fig. 1). Such shoots are etiolated, and differ markedly from the normal adult shoot. It must be pointed out, however, that in propagation of the potato, when tuber pieces are planted several inches below the soil surface, developing shoots are etiolated. Placing the tuber plugs in a darkened incubator reproduced, in part at least, normal conditions of growth.

No precautions other than washing or brushing soil from the stored tubers were taken to prevent fungal contamination of the plugs, but so long as these were cut only from firm, healthy tubers such contamination was slight. Only occasionally were mites observed on shoots growing in the incubator, and it was then found beneficial to keep a small tube of paraffin oil (kerosene) in the incubator.

Satisfactory fixation of material was obtained with a few fixatives only, the most consistent results following the use of formalin-acetic-alcohol. Dehydration was carried out in an ethyl alcohol-xylol series. The shoots were embedded in paraffin or rubberized paraffin and usually sectioned at 6-8 μ in the apical region, and 10-20 μ in more mature parts. Sections were stained in Delafield's haematoxylin and safranin or in the safranin-tannic acid-iron alum stain described by Sharman (1943). This latter stain combination, from which the Orange G was omitted, was useful in permitting identification of phloem sieve tubes which were stained an intense blue-black contrasting with the pinkish colour of contiguous parenchyma cells.

External Morphology of the Shoot

The potato tuber is a swollen, subterranean axillary shoot, the leaves of which are reduced to small, often deciduous scales. In the axil of each leaf or leaf scar is a depression, or ' eye ', in which lie several vegetative buds. These are the terminal and lateral buds of a longitudinally compressed axillary shoot (Artschwager, 1924). The central bud is the most prominent, and is usually the first to develop on resumption of growth, vegetative propagation of the species being by means of such axillary shoots.

The first-formed leaves on a tuber shoot are small, simple scales, and so long as shoots are grown in darkness only such leaves are usually formed. The shoot apex is surrounded by about 10 spirally arranged scale leaves, which can easily be dissected away to reveal the shoot apex as a translucent, glistening yellowish or pale-green, slightly domed mound. A new leaf emerges on the flank of the apical mound and occupies a relatively large lateral area of the shoot apex, which is, therefore, of much smaller area immediately after the emergence of a leaf pri-

mordium than just before. Between the emergence of successive leaf primordia the apex increases in size, partly by continuation of growth in the axial direction, for early in the plastochron the apical smumit rises only 10 μ above the axil of the youngest leaf, but by the end of the plastochron this distance is increased to 20 μ, and partly by lateral growth of those flanks of the apex which will subtend the next 2 leaf primordia to appear. Such lateral protrusions of apical tissue form the foliar buttresses (Grégoire, 1935; Louis, 1935).

The changes which occur in apical morphology during one plastochron are illustrated in Figs. 2-6. The apex in Fig. 2 is at maximal area immediately before leaf emergence. The next leaf primordium to appear, here called I_1, will emerge above and between P_2 and P_3, the second and third youngest leaves respectively, and its future position is already indicated by the broad lateral expansion of the apex as the I_1 foliar buttress between these 2 primordia. The buttress which will subtend the leaf I_2, the next but one to emerge, already forms a smaller, but fairly distinct, projection between P_1 and P_2. Following the emergence of a leaf primordium the area of the shoot apex is considerably decreased (Fig. 3). The shoot apex and newly emergent leaf primordium constitute separate centres of growth, and in that part of the apex which separates them the rate of growth has evidently decreased, resulting in the slight depression seen between the 2 growth centres. This depression, which marks the position of the leaf axil, will be occupied by the future axillary bud. In Figs. 4-6 the gradual increase in area of the apex which causes the return to maximal area is seen to result from growth of the foliar buttresses which will subtend the next 2 leaves to appear.

It is evident from Figs. 2-6 that a foliar buttress begins to grow more rapidly than adjacent interfoliar flanks of the apex during the 2 plastochrons preceding the emergence of a leaf at its summit, and may therefore be observed as a lateral protrusion of the apical surface having a maximum vertical height of 2 internodes. In Fig. 2 the I_1 buttress, which has attained its maximum dimensions prior to leaf emergence, extends as a lateral projection down the apical flank to the level of P_2, the smaller I_2 buttress may be distinguished as a projection of the apical flank to the level of P_1.

A new leaf emerges vertically at the summit of a foliar buttress and overtops the shoot apex early in its first plastochron. The leaf primordium, which emerges as a hemispherical mound (Fig. 3), soon becomes tangentially elongated. During this time the adaxial face which was initially convex becomes concave. Successive leaves arise with an angular divergence of approximately 138°, and occupy a space on the shoot apex above and between leaves which are respectively 2 and 3 plastochrons older. The phyllotaxis is $\frac{2}{5}$ or 2 + 3 (Church, 1904), and in the 6 varieties examined there were approximately equal numbers of shoots with right and left spiral phyllotaxis.

As the leaf grows in length, curving over the shoot apex, its early tangential elongation is further accentuated by continued lateral growth of the margins, giving rise to the lamina, which in scale leaves is usually of simple outline. Lateral leaflets are of rare occurrence in shoots grown in the dark, and there is no distinct petiole. Leaves reach their full height of 2-4 mm when they are about 12 plastochrons old. They do not become reflexed from the bud but typically remain more or less vertical and pressed against the stem. In contrast to shoots grown in the light only very few hairs are produced on dark-grown shoots. A few multicellular hairs first appear in a median position on the adaxial surface of a leaf 3-4 plastochrons old; none are produced from the abaxial leaf surface or from the stem.

In the axils of the 4-5 youngest leaf primordia there is no external evidence of a bud, but on the stem surface in the axils of progressively older leaves there develops a tangentially elongated ridge of translucent meristematic tissue. This is the apex of the axillary bud. The first 2 leaves to emerge on the axillary bud are small prophylls which lie at either end of the elongated meristem, and are at right angles to the axillant leaf. The

FIG. 1 — An etiolated juvenile shoot of potato var. Arran Banner, grown in darkness for 7 days at 25°C. × 1.

third leaf usually emerges on that flank of the bud apex which lies closer to the axillant leaf and always lies closer to the older prophyll. Later leaves of the axillary bud emerges in spiral sequence, and on any one plant axillary shoots with right and left spiral phyllotaxis may both occur.

The distal 3-5 mm of the stem is circular in section and relatively slender, but this gives way to a broader basal region (Fig. 1). At the level of this transition important changes in shoot development occur. The further growth of axillary buds, which above this level had formed 6-8 leaves, is inhibited, and several adventitious roots break through the stem surface just distal and lateral to each leaf.

The conspicuous wings which in the adult shoot extend down the stem from the margins of the leaf bases are absent from the juvenile shoot.

The Apical Meristem

The structure of the apical meristem varies in the different varieties studied. An account will first be given of the meristem in the variety Kerr's Pink and comparison with the apex in other varieties will then be made.

STRUCTURE OF THE MERISTEM — The superficial layer of cells in the shoot apex is shown in transverse section in Fig. 8. The cells are histologically similar, but those situated at the distal extremity are slightly larger and less uniform in shape than cells lying further down the apical flanks. On the flanks of the apical mound the cells tend to lie in rows which radiate from the summit of the apex. This is especially apparent above the axils of the youngest leaves (Fig. 8b). The uppermost 5 layers of cells in the shoot apex are densely staining and meristematic (Fig. 10). Together these 5 layers comprise the apical meristem. The cells of these layers are histologically similar, they are without obvious vacuoles, and the nucleus of each is very large. In each of the apical layers the centrally placed cells are usually slightly larger than those more laterally placed. In the 2 outer layers of cells, divisions at the tip of the shoot are restricted to the anticlinal plane; these 2 layers, therefore, constitute the tunica (Schmidt, 1924). Underlying the tunica are 3 layers of meristematic cells in which some periclinal divisions occur during shoot growth. These 3 layers, which thus show genetic interdependence, together constitute the corpus. Within the corpus, however, divisions in the anticlinal plane predominate and the entire meristem is usually in a highly stratified condition (Figs. 10, 11). Periclinal divisions occur regularly in at least the 2 lower layers of the corpus, and result in periodic obliteration of the stratification in this part of the meristem. Such changes in depth of apical stratification occur in cycles which coincide with changes in the growth rate of subjacent maturing pith cells, and with the development of new leaf primordia on the apical flanks. In order to understand more fully the structure and growth of the meristem it is necessary to examine this relationship more closely.

MERISTEM GROWTH — At a time of maximal stratification in the meristem, recent periclinal divisions are absent from all 5 layers of cells which comprise the apical meristem. Subjacent to the meristem, and contiguous with the lowest layer of corpus cells, lie file meristem cells

(Fig. 10). There is no intervening zone of irregularly arranged cells comparable to the subapical initials which have been described for the apices of many angiosperms (Popham, 1951). The first indication of a return to minimal stratification in the meristem is the occurrence of periclinal divisions, first in the centrally placed cells of the lowest layer of the corpus (Fig. 14), then throughout this layer. Periclinal divisions subsequently occur also in one or more centrally placed cells of the middle layer of the corpus (Fig. 9), but in the superficial layer of the corpus periclinal divisions do not usually occur at this time, the cells continuing to divide strictly in the anticlinal plane. Early divisions in cells derived from the lowest layer of the corpus occur in various planes, but soon the cells begin to vacuolate, and divisions become progressively restricted to the horizontal plane, these cells becoming the file meristem at the summit of the pith.

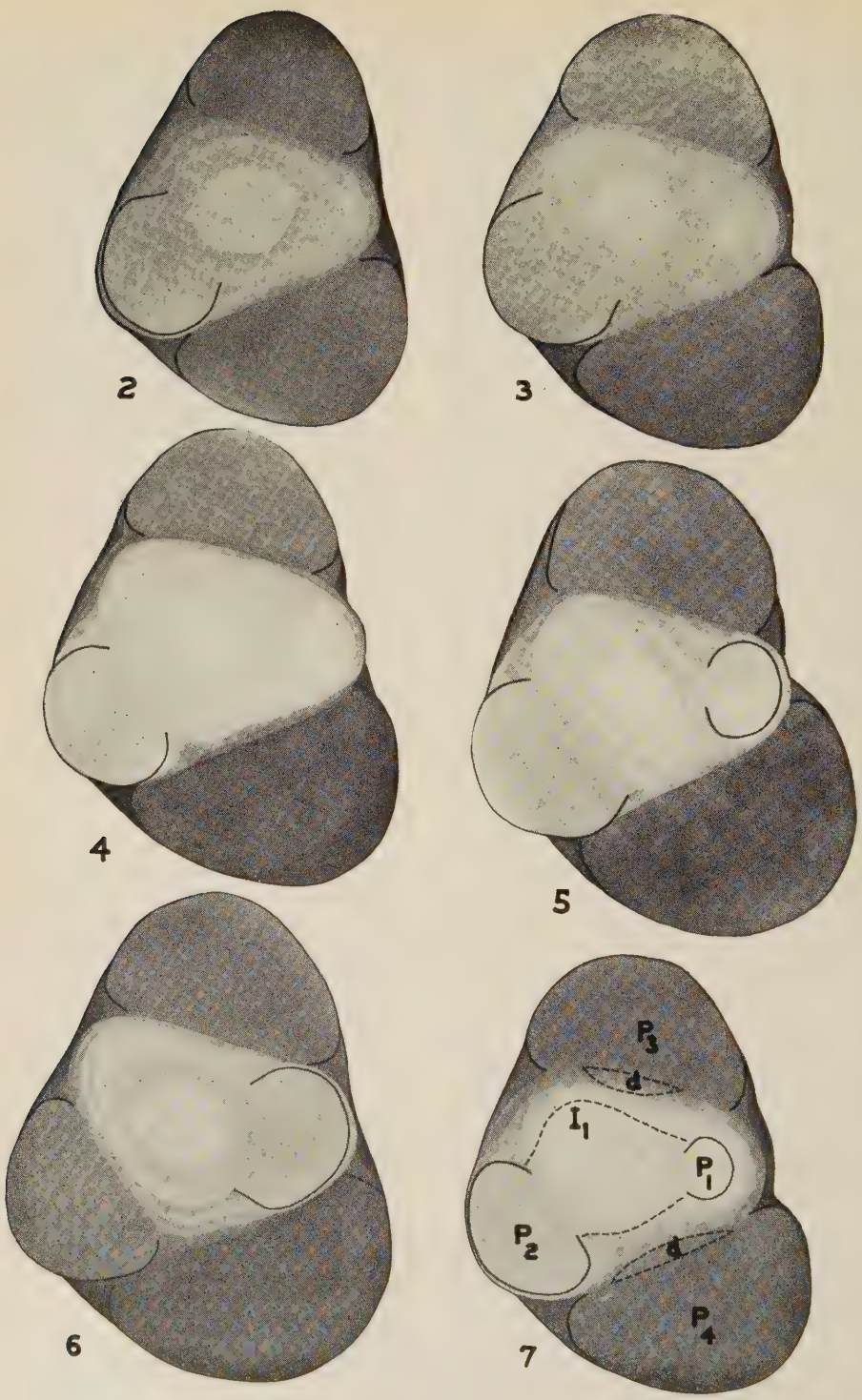
Cells of the lowest corpus layer do not remain permanently meristematic, but all undergo differentiation as pith. They are, however, included as part of the apical meristem because in many features of growth and histology they resemble more closely the other 4 apical layers than the differentiating subapical tissue. The differentiating lowest layer of the corpus is replaced by cells derived from the lower sister cell or cells of the periclinally divided cell group in the middle corpus layer. At the time of this replacement there is in the meristem a central group of 5 superimposed layers of cells which is laterally contiguous with the 4 uppermost layers of cells of the previous stratification cycle (Fig. 9). The maintenance of meristematic activity in the apex is ultimately dependent on the continued growth of these centrally placed cells. In their derivatives, new walls are at first restricted to the anticlinal plane, and by the gradual lateral spread of new cells the meristem returns to a condition of maximal stratification in which 5 superimposed layers of meristematic cells may be distinguished. During the later stages of a stratification cycle in the meristem the subjacent file meristem becomes more clearly defined, as divisions in the youngest cells become progressively restricted to the horizontal plane, and by the time maximal stratification is again approached in the apical meristem the file meristem abuts directly onto the lowest layer of the corpus.

The stratification cycle within the meristem is also related to the stage of the plastochron. Soon after the emergence of a new leaf primordium the meristem is at a stage of minimal surface area and internal stratification is also minimal, but late in the plastochron when the apical meristem is at a stage of maximal surface area all 5 apical layers are stratified.

Although periclinal divisions have not been observed in distal cells of the superficial layer of the corpus, it is considered to form part of the corpus because during early growth of axillary buds only the 2 superficial layers of the meristem are stratified, the cells below these dividing in various planes, and in occasional fully grown apices periclinal divisions have been observed in lateral cells of this layer which lie within the apical meristem. It is thus evident that in the apical meristem of the juvenile shoot of potato there is not a sharp distinction between tunica and corpus.

Lateral Extent of the Meristem — Some distance down the flanks of the apical mound lateral cells of the apical meristem lose their potentiality for unlimited growth and undergo differentiation as permanent tissues of the stem, the transition between the apical and subapical regions of the shoot being marked by a change in the characteristic plane of division in cells lying on the apical flank.

In the superficial cell layer divisions normally occur in the anticlinal plane throughout the duration of shoot growth, but in all underlying layers of cells periclinal divisions occur on the flanks of the apical mound. Derivative cells of the second tunica layer differentiate as the outer part of the cortex, and the position at which the first periclinal division occurs in lateral cells of this layer may be used to identify the margin of the apical meristem. Periclinal divisions in cells derived from this apical layer occur on all flanks of the apical mound; they are not restricted



Figs. 2-7.

to regions of leaf initiation. From examination of longitudinal sections cut in various planes it has been determined that cells of the underlying tunica layer retain their distinctive appearance in the I_1 position and into the axils of the 2 youngest leaf primordia, P_1 (Fig. 10) and P_2 (Fig. 11). In the axils of P_3 and older leaves a small group of distinctive meristematic cells persists, but some distance above the leaf axil periclinal divisions occur in cells of the second tunica layer. Only a few such periclinally divided cells separate the meristematic cells in the axil of P_3 from those of the apical meristem (Fig. 13), but above the axil of P_4 and older leaves the intervening zone of differentiating cortical cells becomes progressively larger (Figs. 12, 13).

The margin of the apical meristem thus delimited around the shoot apex is indicated by the broken line in Fig. 7. The apical meristem is not symmetrical, but meristematic cells extend further down the apical flanks in the I_1 position and into the axils of P_1 and P_2 than in adjacent interfoliar areas. Lying in the axils of P_3 and P_4, and separated from the apical meristem by intervening zones of differentiating tissue, are tangentially elongated areas of persistent meristematic cells which will become the axillary buds of these leaves.

Apical Structure in Other Varieties — With some modification the preceding description, which applies to var. Kerr's Pink, may be used to describe apical structure in the juvenile shoots of the other varieties which were studied. There is a considerable range in the size of the apex in different varieties, which varies from 70-80 μ in diameter at the level of the axil of the youngest leaf primordium in var. Majestic, through 140-160 μ in vars. Kerr's Pink and Ulster Chieftain, to 180-200 μ in vars. Arran Banner, Arran Peak and Stormont Dawn (Fig. 19). In all varieties the size of tunica cells is approximately equal, larger apices having a greater number of cells. In var. Majestic only 4 stratified layers of cells were observed in the meristem. The outer 2 layers comprise the tunica, below which there are 2 stratified corpus layers. Periclinal divisions occur frequently in cells of both the corpus layers. Below these 4 stratified layers is a small group of cells in which the plane of division is irregular and from which the file meristem differentiates (Fig. 20).

In var. Stormont Dawn 5 stratified layers of cells are usually present in the meristem, but in this variety, as in others which have a meristem of large size, there occurs a zone which is absent from the meristem of the other varieties. This is a deep cup-shaped zone of narrow cells extending downwards from near the surface as a curving arc, and continuous below the meristem as the file meristem (Fig. 21). A similar zone of cells occurring in the vegetative shoot apex of *Chrysanthemum* has been described by Popham & Chan (1950). Between the file meristem cells and the lowest stratified layer of the apical meristem in var. Stormont Dawn is a zone of cells in which the plane of division is rather irregular. These cells, which differentiate as the file meristem, are equivalent to the transient

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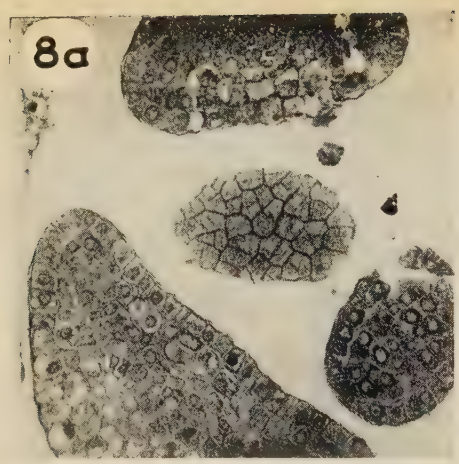
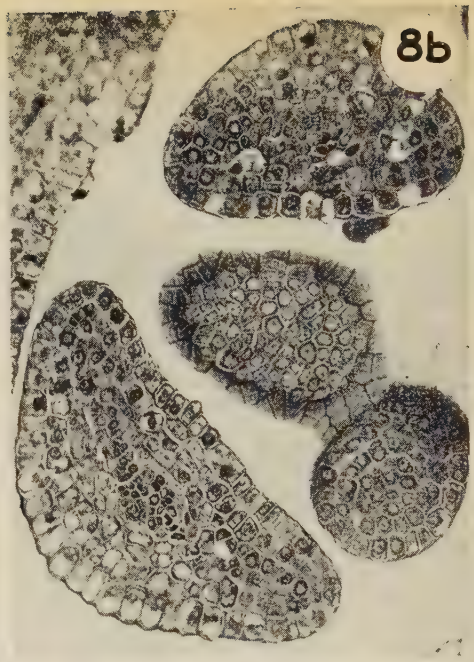
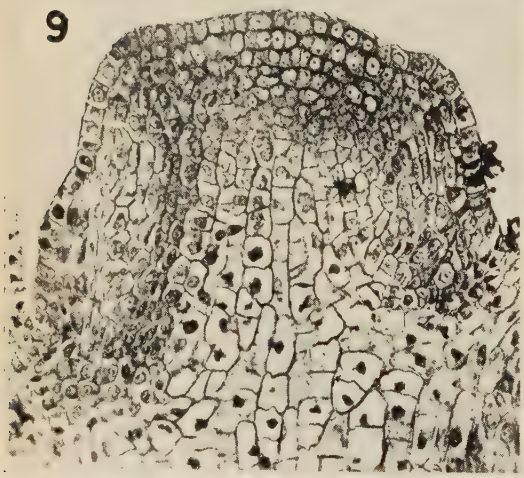
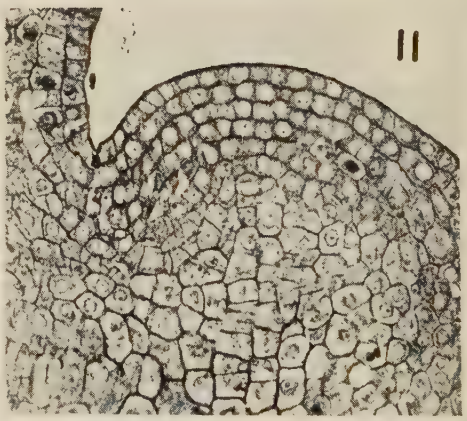
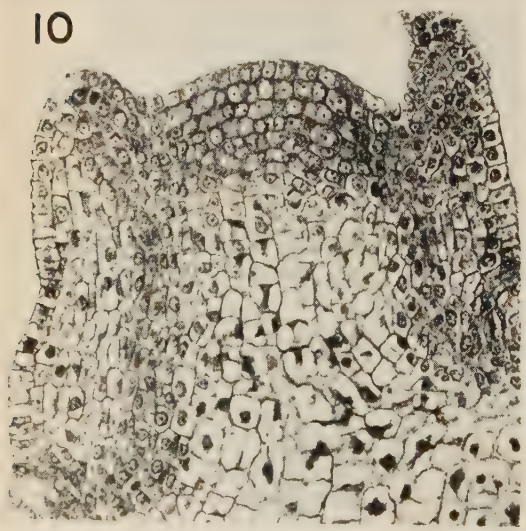
Figs. 2-7 Fig. 2. The shoot apex seen from above at the time of maximal apical area immediately preceding the emergence of a new leaf primordium. The apical mound is asymmetrical as a result of growth of the I_1 foliar buttress. Fig. 3. The shoot apex at the time of minimal area following the emergence of a new leaf primordium at the summit of the foliar buttress on the right of the apex. The buttress which will subtend the next leaf to emerge is already evident as a lateral protrusion of the upper left part of the apical flank. Figs. 4-6. The shoot apex showing progressive enlargement during one plastochron to a new state of maximal area and the gradual assumption of dorsiventrality by the youngest leaf primordium. Fig. 7. The shoot apex seen from above showing the relationship between the apical meristem and the 4 youngest leaf primordia P_1-P_4. The margin of the apical meristem is indicated as a broken line. The positions of detached meristems (*d*) are indicated in the axils of P_3 and P_4. In Figs. 2-6 drawings of successive serial sections, each 18 μ in thickness, are superimposed to give a relief map of the shoot apex. In Fig. 7 successive sections are 6 μ in thickness. Leaves which obscured the apex are shown as cut off immediately above their junction with the stem. Unless otherwise stated these and succeeding illustrations refer to vars. Kerr's Pink. All Figs. × 150.

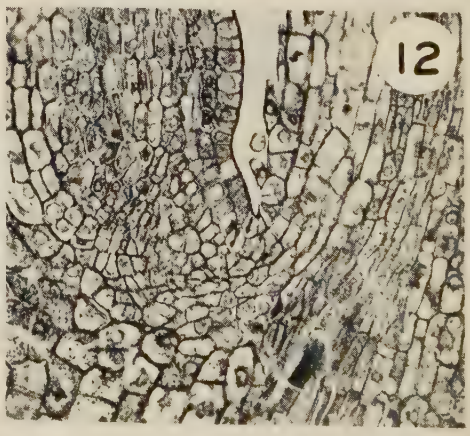












FIGS. 8-12.

irregularly dividing zone which underlies the stratified layers of the meristem in var. Kerr's Pink but in var. Stormont Dawn this zone is persistent.

Tissue Differentiation in the Stem

The initial differentiation of all the tissues of the stem occurs in the subapical region of the shoot, and is indicated in all tissues, except the epidermis, by a change in the plane of cell division from anticlinal, which predominates within the apical meristem, to periclinal.

Cortex — Cortical tissue is derived from the inner tunica and outermost corpus layers of the apical meristem (Fig. 20). Lateral cells in each of these 2 apical layers divide periclinally (Fig. 15), and each cell of the 4-layered cortex again divides in the periclinal plane (Fig. 16). The 8-layered cortex may be distinguished from the internally contiguous residual meristem by the slightly larger size and greater regularity of arrangement of the cortical cells (Figs. 10, 16). Vacuolation first becomes evident in cells of the 4-layered cortex. Vacuolation of cortical parenchyma is accomplished in 2 separate stages. The first is completed early, all cortical cells in the distal 3-5 mm of the shoot being of approximately equal size. Below this terminal region cortical cells undergo further vacuolation, their distension, with that of pith parenchyma cells at this level, contributing to the swollen basal portion of the shoot (Figs. 1, 23).

At maturity the cortex is 8-12 cells in width, the additional cells being derived by further periclinal or oblique divisions in cells of the previous layers. The cortex of the etiolated juvenile shoot is entirely parenchymatous; unlike the adult shoot there is no band of collenchyma (Artschwager, 1918).

Vascular Tissue — The initial delimitation of vascular tissue in the shoot apex and the subsequent differentiation of the first-formed mature elements of the xylem and phloem have been investigated. The direction in which procambial differentiation proceeds has not been studied in detail; however, in a recent review Esau (1954) concludes that the most reliable evidence indicates that differentiation of the procambium in the angiosperm shoot is continuous and acropetal. The term *residual meristem* has been used by Kaplan (1937) and Esau (1954) to describe the vascular tissue in its initial stage of differentiation and will be used here, *procambium* being those parts in the cylinder of residual meristem which become further differentiated as leaf traces.

The initial differentiation of residual meristem in the potato shoot occurs in the subapical region at the margin of the apical meristem. Residual meristem cells are initially no longer than adjacent cells of the pith and cortex, but may be distinguished by the lack of conspicuous vacuolation (Fig. 9). The first periclinal divisions which initiate residual meristem occur in lateral cells of the middle corpus layer of the apical meristem. Subsequent longitudinal divisions in the derivative cells are not restricted to this plane and at an early stage there is no evidence of regularly disposed cell layers such as occur in the cortex (Figs. 10, 16). Because of the rapidity with which successive longitudinal divisions occur the transverse area of individual residual meristem cells is less than that of the cells in the apical meristem from which they were derived, the increasing thickness of the residual meristem tissue as a whole

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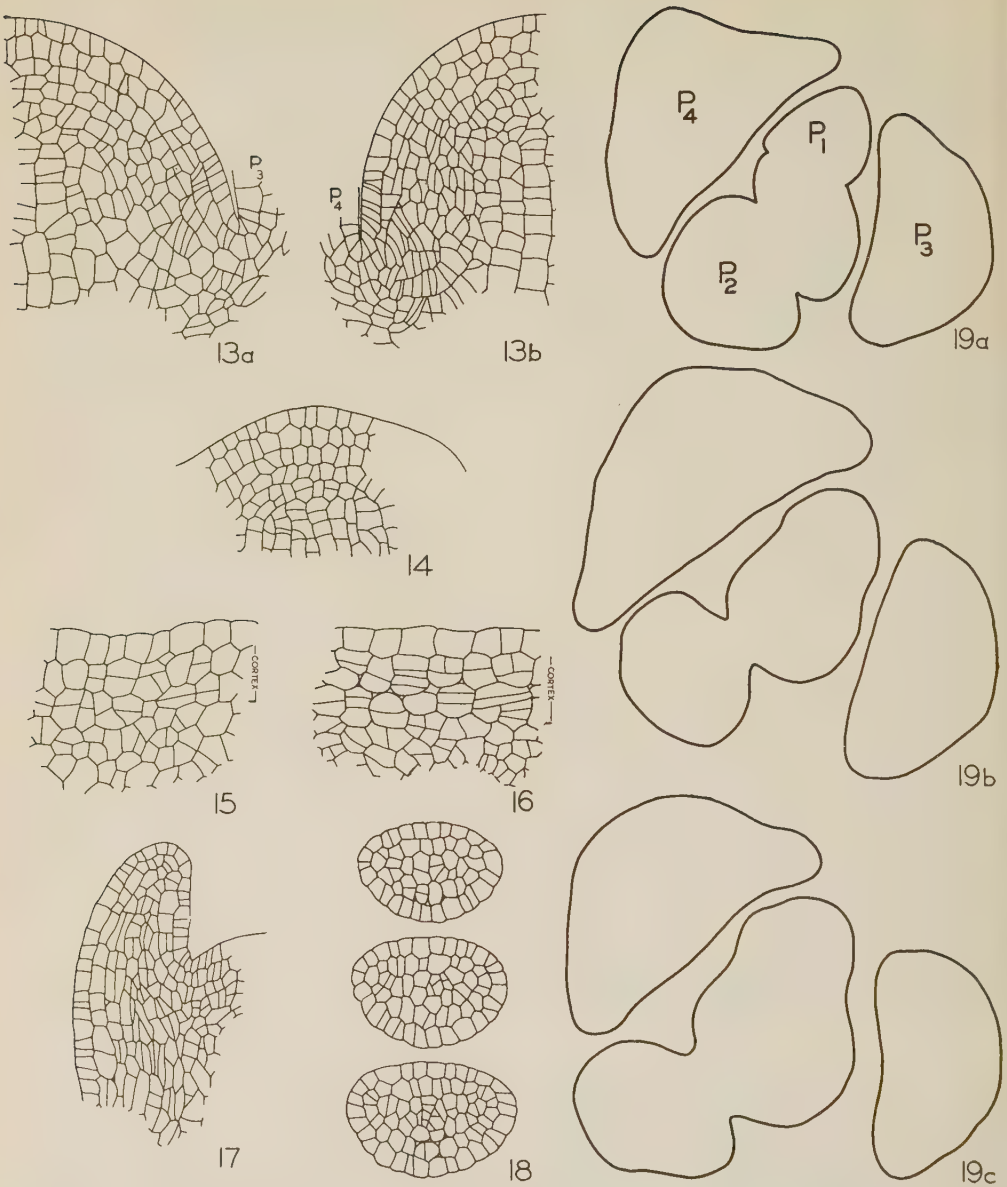
Figs. 8-12 – Figs. 8a and b. Successive t.s. 8 μ thick of the shoot apex showing the superficial cell layer of the apical meristem and the 3 youngest leaf primordia. Fig. 9. Median l.s. shoot apex with a leaf primordium emerging on the left flank. Only the 3 superficial layers of cells in the meristem are stratified. Fig. 10. The shoot apex showing 5 stratified apical layers and file meristem cells contiguous with the lowest layer of the corpus. Fig. 11. The shoot apex sectioned in the plane of P_2, and showing the distinctive appearance of tunica cells in the axil of P_2. Fig. 12. Differentiating cortical cells, derived in part from the underlying tunica layer and in part from the outermost corpus layer, above the axil of P_6 separating the detached meristem immediately above the leaf axil from the apical meristem. All Figs. 225.



FIGS. 13-19 — Fig. 13. L.s. apices through leaves P_3 (left) and P_4 (right). Detached meristems are being delimited immediately above each leaf axil, and are separated from the apical meristem by narrow zones of periclinally divided differentiating cells. × 225. Fig. 14. L.s. shoot apex in which periclinal divisions are occurring in the lowest layer of the stratified corpus. ×225. Fig. 15. T.s. stem in which the initial differentiation of tissues is evident. Incipient cortical cells are derived from the inner tunica and outermost corpus layers of the meristem and are each divided once in the periclinal plane. The 4-layered cortex differs only slightly from adjacent residuel meristem. × 300. Fig. 16. A slightly older stage of tissue differentiation than that seen in Fig. 15 in which the cortex is about 8 layers in depth and now differs from residual meristem in the size and regular arrangement of its component cells. × 300. Fig. 17. Leaf primordium P_1 in l.s. ×150. Fig. 18. T.s. at successively lower levels of leaf primordium P_1 × 150. Fig. 19. T.s. apical meristem of 3 varieties. (a) Majestic. (b) Kerr's Pink. (c) Stormont Dawn. The sections show the stem at the level of the P_2 axil, and the 4 youngest leaf primordia are drawn. × 150.

resulting from increase in cell numbers. The differentiation of residual meristem at the margin of the apical meristem is most easily followed in interfoliar areas of the shoot apex where its ontogeny has not been modified by developing leaf primordia.

Coincident with the emergence of a leaf primordium, procambium is differentiated from residual meristem in the subjacent part of the stem, and may be distinguished by the smaller diameter and more intense staining of the procambial cells. Because of the proximity of the youngest leaf primordium to the summit of the shoot apex, and the early development of procambium below a newly emergent leaf, a continuous ring of residual meristem does not occur in the juvenile shoot. However, in the ontogeny of an apical flank from which a foliar buttress, and subsequently a leaf develop, a residual meristem stage of vascular development precedes the differentiation of procambium. Thus the prevascular tissue of interfoliar flanks of the shoot apex is present as residual meristem while that of laterally adjacent flanks which subtend emergent leaf primordia is at the procambial stage of development. On further vacuolation in the pith and cortex, the cylinder of prevascular tissue becomes more clearly delimited. This cylinder is not of uniform thickness, regions of leaf trace procambium being relatively wide, intervening areas in the cylinder being thinner. Parenchymatization occurs in the innermost cells of the prevascular cylinder giving rise to a tissue indistinguishable from the pith. It is thus not possible in the mature shoot to determine exactly the inner limit of tissues which were derived from the residual meristem.

The earliest vascular elements to mature in the stem behind the apical meristem are phloem sieve tubes situated at the external border of the vascular cylinder in procambium of the median leaf trace below a leaf 4-5 plastochrons old. Differentiated phloem sieve tubes may be externally contiguous with cortical parenchyma cells (Fig. 22); pericycle parenchyma and phloem fibres described by Artschwager (1918) are absent from the juvenile shoot of the varieties studied here. In the 2 lateral leaf traces phloem elements are differentiated 1-2 plastochrons later, and differentiation in the anodic trace is usually in advance of that in the cathodic leaf trace. In each of these 3 traces there are at maturity only 2-3 sieve tubes at any level in the stem. Further behind the shoot apex sieve tubes are found to occur in procambium subjacent to leaf primordia only 2-3 plastochrons old. Differentiation of phloem sieve tubes is continuous and acropetal in direction, and phloem elements first differentiate in a leaf 4-5 plastochrons old. The phloem which supply both the median and lateral traces on a leaf n arises as a single branch, which bifurcates at higher levels in the stem, from the anodic lateral trace of leaf $n + 3$. In *Nicotiana tabacum* Esau (1938) found interconnection between the phleom traces of leaves separated by 3 internodes.

The phloem which lies on the abaxial face of the vascular tissue in the leaf is ultimately continuous with sieve tube strands which lie on the *inner* face of the vascular cylinder in the stem. At some point between its origin from an older trace and its entry into the leaf the phloem trace ' passes ' from the inner to the outer side of the vascular cylinder. Passage of the phloem trace through the vascular cylinder occurs in variable positions not related to leaf gaps, the phloem trace passing directly through the cylinder of procambium as a row of short radially elongate sieve tubes (Fig. 22). This may occur before or after separation of one or both of the lateral traces from the median trace, thus there may be passage of 1-3 phloem traces through the vascular cylinder for each leaf. Other strands of phloem sieve tubes are differentiated on the inner side of the vascular cylinder soon after the appearance of external phloem elements. Some of these lie on the inner face of leaf traces and are continuous into the leaf where they lie on the adaxial face of the vascular tissue, the leaf traces being bicollateral. Internal phloem strands anastomose in the stem in a complex and irregular pattern which is not evidently related to the position of leaf traces. Internal phloem strands may become quite widely separated from the remainder

of the vascular cylinder by parenchymatization of intervening cells; such isolated ploem strands are, however, vascular in origin. They are not medullary phloem as was suggested by Venning (1949) for internal phloem of the tomato shoot.

Differentiation of lignified protoxylem elements in the stem commences later than that of the external phloem in the same trace, and usually is delayed until after the internal phloem of the trace has begun to differentiate. Lignified cells first occur as a discontinuous strand in the median procambial trace in the stem some distance below a leaf 3-4 plastochrons old, and are differentiated in the leaf 1-2 plastochrons later. In the 2 lateral traces xylem is discontinuous at its inception and does not differentiate in the leaf until this is 8-10 plastochrons old. Infrequently, there is no lignification in one or both lateral traces. At maturity there are usually not more than 8 lignified cells at any level in the median trace, and only 2-3 in the lateral traces. The mature vascular cylinder of the juvenile shoot is solenostelic and is composed largely of elongate parenchyma cells, only few lignified xylem elements, and phloem sieve tubes being differentiated (Fig. 24). Cambial activity is slight in juvenile shoots grown in the dark. A cambium becomes functional only in the leaf trace bundle, producing a few secondary vascular elements.

Artschwager (1918) has described the structure of, and the course followed by, leaf traces in the adult stem of the potato. The juvenile shoot differs considerably from his description. Artschwager (1918) holds that in both adult and juvenile shoots differentiation of protoxylem precedes that of external phloem, but in the present study external phloem sieve tubes were consistently found to differentiate in advance of xylem elements in the same vascular strand. The first appearance of internal phloem either preceded or followed the inception of lignification in xylem cells. The accuracy of Artschwager's observation has been questioned by Esau (1938), and it may be mentioned that in the present study reliable information could only be obtained from material stained in a modified safranin-tannic acid-iron alum stain (Sharman, 1943). With other stain combinations it was difficult to distinguish early-formed phloem elements from adjacent parenchyma.

In the adult shoot the lateral leaf traces are more prominent than the median trace, but in the juvenile shoot the median trace is the more prominent. In addition to the scarcity of differentiated xylem and phloem elements, the juvenile shoot differs from the adult shoot in the frequent establishment of continuity between the internal and external phloem systems. For the adult shoot Weiss (1883) found that these were confluent only in the smaller leaf veins, and Artschwager (1918) described fusions at the lateral margins of the petiole bundle. Evidently the pattern of phloem fusion changes during development of the shoot, and in a preliminary examination of the course taken by phloem traces in the adult shoot in several varieties of potato, no confluence has been found to occur in the stem between internal and external phloem. The course of the leaf traces in the adult shoot follows a regular pattern and all primary xylem can be related to the leaf trace system (Artschwager, 1918). It has not been possible to study in detail the pattern of xylem fusions in leaf traces of the juvenile shoot, because of the presence in the stem of supernumerary xylem elements which occur in strands between the leaf traces, but do not pass into the leaves. The supernumerary strands are basally continuous and are differentiated acropetally late in the ontogeny of the stem. Similar acropetally developing xylem traces have been described in early stages of development of axillary buds in other species of angiosperms (Garrison, 1949 a, b).

Pith — The initial differentiation of the pith occurs immediately subjacent to the apical meristem. The pith is derived from cells of the lowest layer of the corpus which undergo periclinal divisions following a period of maximum stratification in the apical meristem. The planes of the first few divisions in the derivative cells are irregular, and a mass of non-vacuolated dividing cells is produced below the apical meristem (Fig. 9). In some varieties at least this zone is tran-

sient, and by the gradual restriction of cell divisions to the horizontal plane a file meristem becomes established at the summit of the pith, and from this pith cells are differentiated (Fig. 10). The pith is composed of longitudinal files of parenchyma cells, and as in the cortex, vacuolation is accomplished in 2 separate stages. The first is completed close behind the apical meristem, all pith cells in the distal 3-5 mm of the shoot being of approximately equal size. Below this terminal part of the shoot, pith cells undergo further vacuolation, their distension contributing to the swollen basal portion of the shoot (Figs. 1, 23).

The Leaf

The emergence of a leaf primordium at the shoot apex is preceded by the lateral expansion of the subtending apical flank as a foliar buttress (Figs. 3-6). Initially, the differentiation of tissues on this flank is not in advance of that which is occurring at the same level on other flanks of the apex; the cortex is 4 layers in width, and in the residual meristem the first few longitudinal divisions have occurred. Residual meristem may be distinguished from internally contiguous pith by the precocious vacuolation of cells in the pith, but the distinction between residual meristem and cortex is at first less evident. Increased cell division on the flank which is to subtend the next leaf primordium to appear first becomes evident in the cortex, where the 8-layered state is attained precociously (compare right and left flanks of the apex, Fig. 9). Vacuolation of the daughter cells further emphasizes the distinction between cortical and residual meristem tissues even before emergence of the leaf.

The increasing thickness of the cortex is in part responsible for lateral protrusion of the foliar buttress, but it is in the deeper-lying tissues, and more especially in the pith, that lateral growth is most evident. On interfoliar flanks of the apex, files of pith cells converge distally towards the lowest layer of cells in the corpus of the apical meristem (right side of pith, Fig. 9), but on a flank undergoing development as a foliar buttress, files of pith cells *diverge* from the centre of the apical meristem, and cells lie in short files which are orientated towards the summit of the buttress (left side of pith, Fig. 9).

The emergence of a leaf primordium follows divisions in several of the more deeply situated layers of cells which occupy a small terminal part of the foliar buttress. At the summit of the buttress periclinal divisions in cells of the second layer of the tunica may coincide with leaf emergence (Fig. 9), but they are often delayed until the primordium has formed a small hemispherical mound of tissue (Fig. 10). Although divisions which bring about the emergence of a leaf primordium are restricted to a relatively small area, it is not from these cells only that the leaf is derived, for as the mound of primordial leaf tissue enlarges it encroaches laterally and distally over the apical surface, incorporating into itself tissues which were previously located at some distance from the centre of leaf inception.

A newly emergent leaf primordium usually stains more intensely than the cells of the apical meristem, and is subtended by a densely staining procambial strand (Fig. 9). Coincident with the emergence of a leaf primordium, cells situated just distally to the enlarging mound of primordial tissue and which were derived from the middle layer of the corpus undergo a succession of periclinal divisions, giving rise to short, outwardly directed files of cells which are continuous basally with the curving files of pith cells which underlie the foliar buttress (Fig. 10). These cells subsequently vacuolate and mature as leaf gap parenchyma.

A newly emergent leaf primordium is nearly circular in transverse section, slightly older primordia are oval, the abaxial surface being rather more convex than the adaxial surface. As the leaf develops further, differences in shape between the two surfaces become greater. The abaxial surface remains convex, but the adaxial surface first becomes flattened, then concave as the leaf extends laterally around the apical flank (Fig. 8). In a very young primordium, which is still circular in outline, there are equal numbers

of cells along the radial and tangential axes, but as dorsiventrality becomes more evident the number of corpus-derived cells increases more rapidly along the tangential axis of the leaf than along the radial axis (Fig. 18). Thus the initial dorsiventrality of a leaf primordium is the result of a more rapid rate of cell multiplication along the tangential axis.

Cells derived from the corpus comprise much of the bulk of a newly emergent leaf primordium, derivatives of the tunica remaining as mantling layers. It is possible to establish approximately the initial contribution made to the leaf by each of the layers of cells of the apical meristem. Near the base of the leaf primordium the 2 outermost layers of cells are devoid of periclinal divisions (Fig. 18). These layers are continuous with the tunica layers of the apical meristem (Fig. 17). The internal tissue of the leaf is derived from the 2 outer layers of corpus cells, but the boundary-separating tissue derived from each of these apical layers is not distinct. In the margins of a young primordium, cellular configuration is such that only the 2 tunica layers and the outermost layer of the corpus can have contributed cells (Fig. 18). Cells derived from the middle layer of the corpus are apparently confined to the thicker central part of the primordium. Nearer the summit of the primordium the internal tissue is less extensively developed; close to the tip corpus derivatives are only 2 cell layers in width, and are thus all produced from the outermost layer of the corpus, the middle corpus layer not being represented at this level (Fig. 18).

A newly emergent leaf primordium consists of a mound of meristematic tissue situated at the summit of a foliar buttress which is already relatively highly vacuolated. Vacuolation in the cortex approaches closest to the shoot apex in the foliar buttresses, cells of adjacent interfoliar regions being relatively unvacuolated. From the buttress a wave of vacuolation spreads up the abaxial face of the leaf primordium soon after emergence. Vacuolation on the adaxial face of the leaf is later in inception, and is initially discontinuous both from that of the abaxial side of the leaf, and from vacuolation in the pith. As the leaf gap parenchyma undergoes vacuolation, connection is made between vacuolated pith cells and the adaxial parenchyma in the leaf, but between vacuolated cells on the two faces of the leaf is a persistent arc of non-vacuolated cells which extends between the margins of the leaf (Fig. 8). It is in this arc of residual meristem that leaf procambium is differentiated. Procambium occurs first in the centre of the arc of residual meristem as a small strand of narrow cells (Fig. 18). Differentiation of procambium in the centre of the leaf precedes the complete delimitation of the residual meristem in the leaf margins. Procambium is increased by divisions in contiguous cells of the residual meristem. In the distal parts of the leaf the median procambial strand remains cylindrical in section, but near the leaf base procambium is extended towards the margins of the leaf by the continuation of procambial differentiation laterally through most of the residual meristem. Procambium in the proximal part of the leaf is, therefore, crescentic in section.

The initial dorsiventrality of the leaf primordium becomes increasingly evident as the leaf enlarges. Even before the marginal meristems begin to function, the leaf is dorsiventral because of the early tangential growth of cells derived from the corpus. The result of this is to increase the relative volume of leaf tissues derived from the deeper layers of the apical meristem. The mature lamina does not then consist exclusively of tissue derived from the tunica layers in the meristem, but tissue adjoining the median vascular strand in the proximal parts of the leaf bears a genetic relationship to the more deeply situated apical layers.

The submarginal initials, which give rise to most of the tissue of the leaf lamina, are lateral cells of the residual meristem, and are derived from the second tunica layer in the meristem. The sequence of divisions in these initials is not as clear as that described for *Nicotiana* (Avery, 1933), but the inner periclinal derivatives contribute to the deeper layers of the lamina. In the thickened leaf base there may be more than a single row of sub-

marginal initials; usually a small group of irregularly arranged meristematic cells occurs.

The earliest axial growth made by the leaf is the result of divisions in deeply-lying cells elevating a mound of tissue at the summit of the foliar buttress, but this is soon succeeded by apical growth of the leaf primordium itself. A small group of 3-6 terminal cells of the subepidermal layer functions as a meristem. The periclinal derivatives of these cells differentiate as the internal tissue of the leaf. Apical growth in leaves of the juvenile shoot is of very short duration, terminating when a leaf is 5-6 plastochrons old. The leaf tip becomes vacuolated and further elongation is the result of intercalary growth.

The rate of leaf development is approximately uniform in the varieties examined, but because apical size varies greatly, the relation of leaf primordium size to shoot apex size differs in each variety. Thus in var. Majestic, which has a small apical meristem, young leaf primordia each occupy a relatively large proportion of the apical flank. In vars. Kerr's Pink and Stormont Dawn, in which the meristem is of larger size, the leaves occupy relatively less of the apical flanks (Fig. 19).

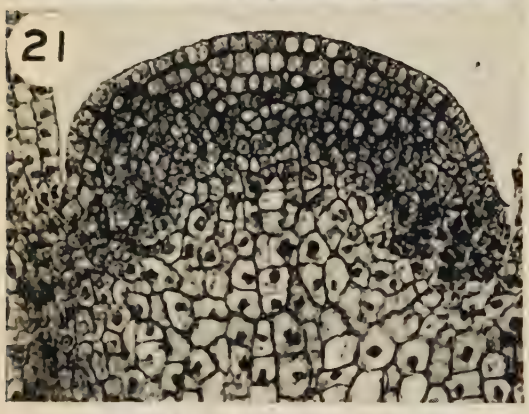
Mature scale leaves of the juvenile shoot are simpler in structure than the leaves of the adult shoot described by Artschwager (1918). Leaf mesophyll is composed of 3-5 layers of isodiametric cells. In the main veins there are more differentiated vascular elements than occur in the leaf traces in the subjacent part of the stem. Internal phloem is differentiated in the larger veins, but not in the smaller ones. The ontogeny of the vascular elements in the leaf will not be reported in detail. There is, however, a general resemblance to that reported by Avery (1933) for *Nicotiana tabacum.*

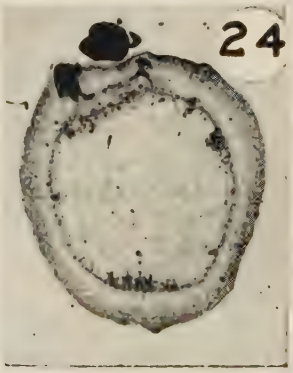
The Axillary Bud

The apical meristem of the shoot extends laterally into the axils of the 2 youngest leaf primordia (Fig. 7). When a leaf primordium is at the end of its second plastochron of growth, cells on the apical flank a little distance above the leaf axil undergo 1-2 successive divisions, the plane of which in superficial layers is approximately anticlinal, but in deeper layers curves down towards the leaf (Fig. 25). Divisions in contiguous vacuolating cells just below the leaf axil complete the delimitation of a small tangentially elongated band of persistent, isodiametric meristematic cells in the leaf axil (Fig. 12). This band of meristematic tissue, which is 4-5 cell layers in depth, and retains the layered appearance of the apical meristem, becomes separated from the apical meristem by the onset of periclinal divisions and differentiation in intervening cells (Fig. 13). Vacuolation of these cells as cortical tissue proceeds in such a manner as to leave the meristematic cells in the leaf axil connected by 2 strands of short, nonvacuolated cells, one of which lies on each side of the leaf gap, to the cylinder of residual meristem. These strands are the bud traces, and connect the axillary bud with the vascular system of the parent shoot.

The meristematic cells delimited in the axil of a young leaf primordium are similar in origin to the detached meristems described from various species of ferns (Wardlaw, 1943a, b) and angiosperms (Garrison, 1949a, b). From them the axillary buds develop. When first delimited the detached meristem is only 4-5 cells wide along the proximal-distal axis of the parent shoot, but along the tangential axis there is a greater number of cells. The detached meristem is, therefore, initially elongated in the tangential direction of the parent shoot (Fig. 7).

A detached meristem begins to grow soon after it is completely delimited. Cells of the deeper layers divide, at first periclinally or obliquely, later in various planes, and the meristem surface bulges outwards. The two superficial layers of cells at the summit of the axillary shoot divide only in the anticlinal plane and constitute the tunica, subjacent cells belonging to the corpus (Fig. 26). As in the apex of the parent shoot the margin of the axillary bud meristem may be delimited by the position at which the first periclinal division occurs in cells of the second tunica layer.





FIGS. 20-27.

As the bud is elevated further from the leaf axil its meristem grows more rapidly in the plane parallel to the proximal-distal axis of the parent shoot, thus reducing the initial asymmetry.

As the diameter of the meristem increases, cells in the superficial layer of the corpus begin to divide more frequently in the anticlinal plane and attain a stratified condition. Subsequent divisions in these cells are restricted to the anticlinal plane (Fig. 27). In still larger apices stratification occurs progressively in underlying cells of the corpus and the structure of the fully-grown stratified meristem is attained. In the apex of an enlarging axillary bud cells in the 2 tunica layers attain the same order of size as comparable cells in a fully-grown apex early in bud ontogeny, but cells of the corpus and the pith are very much smaller in small apices (Fig. 26), and only attain their maximum size as the meristem becomes more highly stratified (Fig. 27).

The bud continues to grow until there are 6-8 leaf primordia surrounding its apex, by which time it occupies a position where extensive vacuolation in the cortex and pith causes a considerable increase in diameter of the parent stem. Below this point its further growth is normally inhibited, and it persists as a short axis in the leaf axil (Fig. 23).

Discussion

Following the description by Schmidt (1924) of apices of several angiosperms in which the terminal meristem could be divided into two separate regions, a superficial layered tunica in which divisions were anticlinal, and an underlying corpus in which the plane of division was usually less regular, a large number of species were found to conform to this general pattern of apical structure (Foster, 1939). Subsequently, it became evident that in many angiosperms the shoot apex could be further subdivided into cytohistological zones which differed in the size, the degree of vacuolation and the frequency of division of component cells (Foster, 1941; Gifford, 1954). Other species have been described in which distinction between tunica and corpus is not constant, in that there may be ontogenetic progression in the number of tunica layers (Kasapligil, 1951; Chakravarti, 1953), a regularly fluctuating number of tunica layers (Cross, 1937; Sterling, 1949; Gifford, 1950), or occasional periclinal divisions in deeper layers of the tunica (Reeve, 1948).

Because of the wide range found to occur in the structure of the shoot apex of different angiosperms, some workers have questioned the general suitability of Schmidt's terminology, and it has recently been suggested (Popham & Chan, 1950; Popham, 1951) that a more accurate description of the terminal meristem results if all the stratified cell layers in which anticlinal divisions *predominate* are grouped together as the mantle, which may thus include the tunica layers and any superficial layers of the corpus in which only occasional periclinal divisions are observed. The subjacent large, often vacuolated, irregularly arranged cells of the corpus are distinguished by these workers as the subapical initials. The deeper mantle layers and lateral cells of the subapical initial group contribute to a densely staining, small-celled, stratified peripheral meristem from which new leaf primordia arise and from which cortical

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FIGS. 20-27 — Fig. 20. Median l.s. shoot apex of var. Majestic. × 225. Fig. 21. Median l.s. shoot apex of var. Stormont Dawn. × 225. Fig. 22. Longitudinal stem section with a phloem strand passing from the inner to the outer face of the vascular cylinder. × 225. Fig. 23. L.s. juvenile stem showing the slender distal and broad basal regions. In the axil of a leaf on the left side of the stem is a short axillary shoot. × 7. Fig. 24. T.s. juvenile stem in the mature basal region. The vascular tissue forms a complete cylinder which is only a few cells wide except in the leaf traces. An axillary shoot, and adventitious roots are present at one side of the stem. × 7. Fig. 25. The initial delimitation of detached meristem cells in the axil of P_3. × 225. Fig. 26. Early axillary bud growth. Only the 2 tunica layers in the meristem are stratified (var. Stormont Dawn). × 225. Fig. 27. An older axillary bud than that in Fig. 26 in which the outermost corpus cells are becoming stratified. × 225.

and procambial tissues are differentiated. Immediately below the subapical initials lies the central meristem which gives rise to the pith by transverse divisions of its cells. Substantial agreement with this account of apical zonation is reached by Philipson (1954) who recognizes only 3 zones in the meristem, considering that the peripheral zone and central zone (which includes the subapical initials of Popham's description) extend to the surface of the apex, and thus include also the anticlinally-dividing cell layers of the tunica.

The terminal meristem in the juvenile shoot of the potato-variety Kerr's Pink does not conform closely to any of the above descriptions of apical structure, and is evidently an extreme example of periodic stratified growth involving all layers of the apical meristem. When apical stratification is at a maximum there is no zone of subjacent irregularly arranged cells, but contiguous with the lowest of the 5 stratified apical layers are longitudinal rows of file meristem cells which lie at the summit of the pith. A zone of irregularly arranged cells intervenes between the stratified apical meristem and the file meristem only in the early stages of each plastochron. This zone is derived from the lowest layer of corpus cells during a period of minimal stratification in the meristem, and during the later stages of each plastochron all of its component cells differentiate as file meristem. It is doubtful that this zone of cells is equivalent to the subapical initials of other angiosperms because it is transitory in occurrence, the component cells have a high rate of division in contrast to the low rate of division stated to occur in subapical initials of other species (Popham, 1951), and differentiate entirely as pith. For these reasons, and because it is not possible to distinguish histologically or cytologically between peripheral and central regions of the meristem, it is considered preferable to retain the tunica-corpus terminology in describing the meristem. A similar lack of cytological zonation in the terminal meristem of several species of *Solanum*, including the potato, was reported by Steinberg (1950), who also observed stratified layers in the corpus of *S. sodomaeum*. Schmidt (1924), Zimmerman (1928) and Lance (1954) have also described varying degrees of stratification in superficial layers of the corpus in other angiosperms, and Schmidt (1924) demonstrated that there was an increase in the number of stratified corpus layers as the apex grew from minimal to maximal area.

In the Solanaceae a 2-layered tunica occurs in *Datura stramonium*, which also possesses a stratified superficial layer in the corpus (Satina, Blakeslee & Avery, 1940), in *Lycopersicon* (Steinberg, 1950) and in several species of *Solanum* (Kruger, 1932; Steinberg, 1950). Only in *S. nigrum*, from which Steinberg (1950) has described 3 tunica layers, have more than this number been found.

Apical structure in the potato has previously been described in detail only by Steinberg (1950), who found a 2-layered tunica. Asseyeva (1931) obtained indirect evidence suggesting that most varieties possessed a 2-layered tunica in the tuber apex, but that some appeared to have a larger number of stratified apical layers. This suggests comparison with the findings of various degrees of corpus stratification in the present investigation. Baker (1943) concluded that there were only 2 independent generative layers in the meristem of a strain in which he had induced periclinal chimeras, and his published illustrations show a single tunica layer with periclinally divided cells in the subjacent layer. A 2-layered tunica is evidently of common occurrence in the Solanaceae, and in some members more than that number of stratified apical layers may exist.

The varieties of potato described in the present study are similar in each possessing a 2-layered tunica and several stratified corpus layers in the apex of the juvenile shoot, but certain structural differences between them suggest a causal relationship between size and structure of the meristem. Thus in the relatively small meristem of var. Majestic there are only 4 stratified layers, and in both the lower 2 layers periclinal divisions are of frequent occurrence. In all those varieties with a meristem of larger size,

5 stratified apical layers occur, and periclinal divisions normally occur in only the 2 lowest layers, the superficial layer of corpus cells having attained a tunica-like condition. The large meristem of var. Stormont Dawn possesses in addition a zone of cambium-like initials (Popham & Chan, 1950) not present in the other varieties, and below the stratified meristem layers a persistent zone of irregularly arranged cells from which the file meristem arises. The meristem of this variety approaches closest to those angiosperm apices which have been described in terms of zonal growth patterns, and is, therefore, of particular interest in indicating the significance of apical structure in the other varieties.

A relationship between size and structure of the apical meristem is further indicated in the changes undergone by an axillary shoot apex during its ontogeny. When the detached meristem in the axil of a leaf begins to grow it is of small size, and possesses a 2-layered tunica, and an underlying unstratified corpus in which the cells are very small and irregularly arranged. During its growth the bud meristem increases in size, and superficial cell layers in the corpus become progressively stratified, until when the meristem has attained its full size there are 5 stratified apical layers. Further fluctuations in apical stratification then occur only in relation to the emergence of new leaf primordia on the apical flank. A decreased number of stratified apical layers has been noted during leaf emergence in other species (Cross, 1937; Reeve, 1942; 1948; Sterling, 1949). In these species the meristem is of small size, and like that of the juvenile potato shoot, is only slightly domed above the youngest leaf primordium, and undergoes a marked decrease in size during leaf formation. Millington & Gunckel (1950) and Popham & Chan (1950) have described species in which the meristem is large and steeply domed above the youngest leaf primordium, the emergence of which would not, therefore, be expected to decrease greatly the total area of the apical meristem. In these species there is no evidence of a decrease in apical stratification during leaf emergence.

It was suggested by Cross (1937) that the decreased apical stratification which occurs during or immediately following leaf emergence could be attributed to a shift in the centre of meristematic activity from the apical meristem to the young leaf, but in view of the evidence presented here, the decreased apical size which results from leaf formation in some species may itself be a factor contributing to the fluctuations in apical stratification.

The apical meristem in the juvenile shoot of potato is remarkably similar in its general surface distribution at the apex to those described by Wardlaw (1943a, b) from several species of leptosporangiate ferns. The meristem does not extend an equal distance down all flanks of the apical mound, but its margin shows irregularities of outline which are related to the positions occupied by leaf primordia. The simpler outline of the potato meristem as compared with those in the ferns may be attributed to the presence of fewer leaves which abut onto the apical meristem in the potato shoot than in the fern shoots. The emergence of new leaf primordia just within the margin of the apical meristem occurs in ferns (Wardlaw, 1949) and in the potato, and in both, detached meristems which were originally portions of the apical meristem develop as axillary buds (Wardlaw, 1943a, b). Despite the evident histological differences between apical meristems of ferns and angiosperms, the organs and tissues produced as a result of their activity are broadly comparable, and it is perhaps not surprising to find an essentially similar distribution of meristematic activity in the shoot apex of both.

On the basis of results obtained by the application of surgical treatments to the apical meristem of various ferns and angiosperms, Wardlaw (1950) has concluded that there appear to be underlying morphogenetic patterns common to all vascular plants. However, there are as yet few data which relate to comparable aspects of morphogenesis in ferns and angiosperms, and while general similarities of development may be recognized, it is not yet clear to what extent these are the result of similar underlying mechanisms. Before an extended comparison

of morphogenesis in ferns and angiosperms can be undertaken, it will be necessary to accumulate, particularly for the angiosperms, further data along lines already suggested by the experimental studies of Snow & Snow (1931, 1952), Wardlaw (1943a, 1950) and Ball (1948, 1952).

Summary

The juvenile shoot of potato is an axillary bud developed from the tuber. In this study shoots were grown in darkness and were etiolated; the mature leaves were simple in outline and 2-4 mm in length. The shoot apex is 70-200 μ in diameter and 10-20 μ in height in the 6 varieties studied, and attains its maximal area immediately prior to leaf emergence, at which time foliar buttresses subtending the positions at which the next 2 leaves will emerge are already evident as lateral protrusions of the apical surface. Leaf emergence reduces the apical surface to minimal area. A newly emergent leaf primordium is hemispherical, but soon becomes dorsiventral.

In variety Kerr's Pink the apical meristem consists of a 2-layered tunica and a corpus in which there are 3 stratified cell layers at the end of a plastochron. It can be delimited in surface extent by the positions at which periclinal divisions occur in lateral cells of the underlying tunica layer. Early in a plastochron periclinal divisions occur in each of the 2 lowest layers of corpus cells. Apical stratification is thus decreased, but as the apex increases in size, stratification again becomes evident by progressive restriction of divisions to the anticlinal plane in the cells of the apical meristem. With some variations this pattern is repeated in each of the varieties studied.

The cortex is derived from the inner tunica and outermost corpus layers of the meristem; the residual meristem, from which procambium and primary vascular tissues are differentiated, is derived from the middle layer of the corpus; and the pith from the lowest corpus layer. The mature vascular cylinder is solenostelic, and only few differentiated xylem and phloem elements occur.

A leaf emerges as a hemispherical mound. Early growth is more rapid along the tangential axis and the leaf becomes dorsiventral. Dorsiventrality is further emphasized by continued lateral growth of the marginal meristems. Axillary buds arise as detached meristems in the axils of leaves. They initially have a 2-layered tunica and an unstratified corpus, the superficial layers of which become progressively stratified as the bud enlarges.

The relationship between size and structure of the apical meristem, and the general similarities in distribution of meristematic activity at the shoot apex of potato and ferns are discussed.

It is a pleasure to express my gratitude to Professor C. W. Wardlaw for his encouragement and many helpful suggestions. I also wish to thank Mr. E. Ashby for the photographic illustrations.

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OBSERVATIONS ON SOME ABNORMAL FRUITS OF THE TOMATO, *LYCOPERSICON ESCULENTUM* MILL.

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Introduction

In order to discover the factors which bring about the expression of plant form it is necessary to examine the developmental potentialities of all parts of the plant, and in particular of meristematic tissues, by both experimental and analytical techniques. Indeed, the problems of plant morphogenesis are of such diversity and complexity that they must be attacked from many angles, and by the employment of any promising methods or materials which become available. The abnormal tomato fruits which form the basis of this study (Fig. 1) were grown in Leicester by Mr. W. Ward and were sent for examination to this Department by Dr. H. R. Fletcher, then Director of the Royal Horticultural Society's Gardens at Wisley, and in view of the obvious morphological interest of the material a full analytical investigation was undertaken.

Since only one out of one hundred and forty plants grown together under glass showed the abnormal condition, and all the fruits of this one plant were affected, it was at first considered that the abnormality must be of genetic origin. While many genetic variations of tomato flowers and fruits show much less morphological anomaly than the specimens under investigation (e.g. Rick, 1945; Rick & Robinson, 1949, 1951), others bear a considerable resemblance to them (Lesley & Lesley, 1928; Zielinski, 1948). Anatomical investigation of the fruits, however, has yielded evidence which suggests that the abnormality may have been the result of a virus disease. In the absence of fresh material it is not possible to confirm or refute this suggestion with certainty, since transmissibility using infection techniques is the only certain distinction between virus diseases and genetic abnormalities (Bawden, 1950). The grower states that the plants were not treated with growth hormones.

These tomato fruits have yielded much interesting information about the potentialities of floral meristems, the continued growth of the floral axis and the leaf-like character of the carpels being of particular morphological interest. It is necessary, however, to recall the warning of Arber (1950) that while the study of teratisms yields interesting information about the potentialities of the plant, which may throw light on normal forms, such data must not be considered on a phylogenetic basis.

Material and Methods

The material consisted of several trusses of maturing fruit of the variety Sutton's Ailsa Craig, together with parts of the peduncle. None of the vegetative parts of the plant was available for

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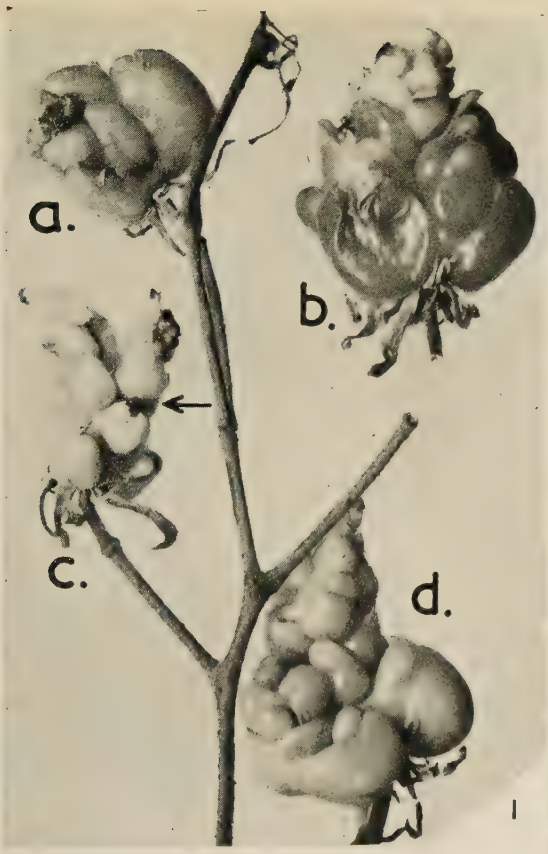
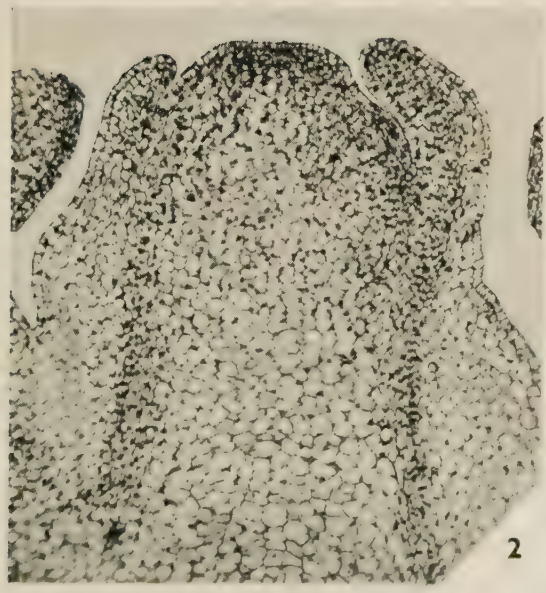
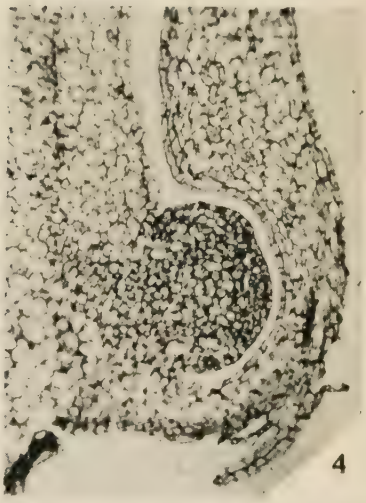
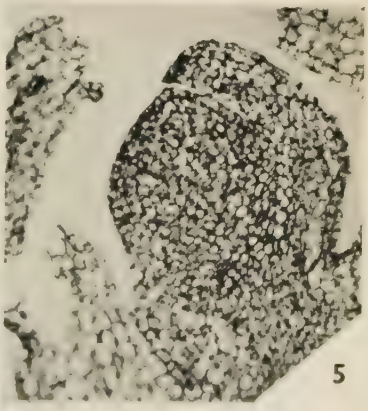
FIGS. 1-5 — Fig. 1. Part of a truss of abnormal fruits. *a*, *b* and *d* show some tendency towards syncarpy; *c* is entirely apocarpous. Note the branching of the axis in *c*. The arrow indicates a lateral axillary axis. × 9/10. Fig. 2. Apex of an abnormal fruit axis in l.s. Prestlar tissue is present in the lateral carpels. × 138. Fig. 3. T.s. carpel near the apex. Meristematic tissue is present adaxially and in marginal positions. × 184. Fig. 4. L.s. carpel with an axillary axis. × 138. Fig. 5. L.s. carpel with an older axillary axis, bearing its first primordium. Tissues of the axillant carpel rather torn. × 138.









Figs. 1-5.

examination, and no early stages of flower or fruit formation were received. Normal material of the variety Ailsa Craig was used for comparison.

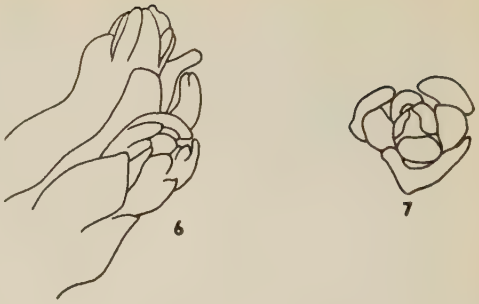
The fruits were fixed whole or in parts in chromacetic and formalin-acetic-alcohol fixatives (Chamberlain, 1926; Johansen, 1940). Sections were stained in Delafield's haematoxylin and safranin (Chamberlain, 1926), and in Feulgen's reagent (Darlington & La Cour, 1947). Microchemical tests were carried out with iodine and Millon's reagent (Chamberlain, 1926).

Morphology

The grower states that the plant grew normally to the same height as the others, but possessed leaves with elongated leaflet stalks, although of healthy appearance.

Each of the abnormal fruit masses had developed from a single flower. At the base of each fruit the remains of a calyx was present, and there was a tendency for the corolla also to be retained in this position (Fig. 1). In normal fruits the corolla falls off soon after pollination (Cooper, 1927); occasionally, however, it appears to be carried up by the developing fruit and shed from the distal end together with the style. Calyces and corollas from the abnormal truss were found to be normal, except for one corolla which consisted of four petals bearing eight stamens, instead of the more usual six petals bearing six stamens.

The abnormal fruits appeared to have been formed by continued growth of the floral axis together with at least partial apocarpy. In some cases (Fig. 1a, d) some union of the carpels had taken place at the base of the fruit, resulting in the formation of a normal fruit wall which later became split and distorted due to continued growth of the axes, but in other cases apocarpy was complete (Fig. 1c). In the apocarpous fruits the adaxial faces of many carpels bore brownish areas of tissue, of a rather corrugated appearance. The tip of the axis was surrounded by many curled styles with small basal carpels; these were arranged spirally around the apex and were of dorsiventral symmetry (Figs. 6, 7).



Figs. 6, 7 — Fig. 6. Two apices which have resulted from bifurcation of the axis, seen from the side, showing the numerous flattened carpels and styles arranged round the apices. × 15. Fig. 7. Apex of a fruit axis from above, showing the spiral arrangement of the dorsiventral styles and carpels. × 32.

Bifurcation of the fruit axis was a general feature of the specimens examined; in some cases this appeared to be a genuine dichotomy, in others the two axes resulting from the division were subequal. Dichotomy was not observed actually at the apex on any occasion. Probably bifurcation is an inherent characteristic of the tomato apex; according to Gorter (1951) the normal inflorescence is formed by repeated dichotomies of the growing apex. Dichotomy of the vegetative axis has also been recorded (Caldwell, 1930; Went, 1944).

Lateral axes in positions axillary to the carpels were also present (Fig. 1 c, arrowed). Dissection of specimens in which a normal fruit wall was present basally, often revealed a lateral carpel-bearing axis within the fruit wall, similar to the main axis of the fruit. In many instances these axes were already branched, and were about to break open the fruit wall. In some cases a hole was present in the outer wall at the point where the internal axis would emerge.

No seeds or ovules were found in any of the fruits examined.

Anatomy

A study of the anatomy of both entirely apocarpous and partially syncarpous fruits and their apical organization served to

confirm and extend the morphological observations. Since fixation of the whole fruits was rather poor, observations were confined in general to gross anatomy and no detailed histological examination was attempted. Fixation of apical parts was quite good, but unfortunately it was not possible to undertake a critical investigation of the differentiation of the primary vascular tissues, since apical material was limited, and the major part was used for longitudinal sectioning.

Apices of the Fruit Axes — Transverse and longitudinal sections of apices dissected out from the abnormal fruits showed that apical organization was apparently quite normal (Fig. 2). Apices were broadly parabolic, as in the normal tomato, and bore lateral organs, in this case carpels. Transverse sections confirmed that these were arranged in spiral sequence and were dorsiventrally flattened. Their striking resemblance to leaves borne on a vegetative apex (*see* Fig. 7) was enhanced by the presence of meristematic tissue on the adaxial face (Fig. 3), which occupied positions similar to the marginal and adaxial meristems in the leaves of tobacco and other plants (Avery, 1933; Esau, 1953). Prestelar tissue was present in the styles and carpels (Fig. 2), and vascular gaps occurred at their insertion on the axis.

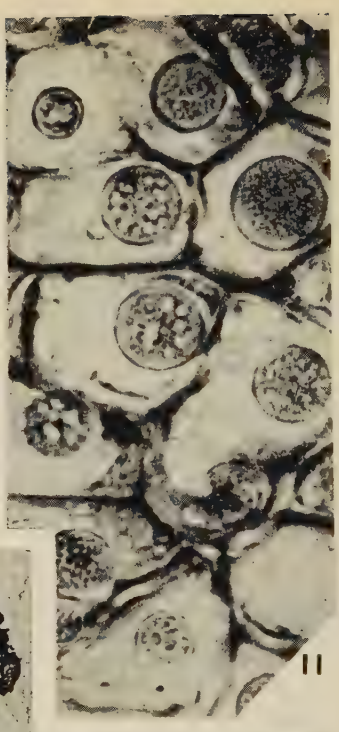
Sections of apical material also confirmed the presence of lateral axes. These may occur in the axils of carpels not far behind the apex (Figs. 4, 5). From observations on external morphology and gross anatomy it appeared that such lateral axes bore only carpels, that is, that they did not pass through the various phases of organogenesis characteristic of a normal floral apex but commenced at the carpellary phase.

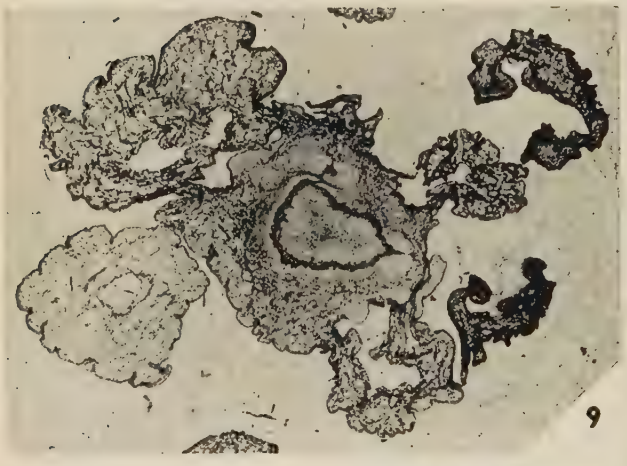
Gross Anatomy — In the development of a normal tomato fruit the vascular supply to each carpel divides, part of it traversing the pericarp into the style and the other part passing into the placenta and there dividing again to supply the ovules (Fig. 8; *see* also Cooper, 1927; Murray, 1945). In the abnormal fruits, however, the vascular supply to the carpels was inconspicuous, except near the apex, and a vascular cylinder was present in the central axis (Figs. 9, 13-22).

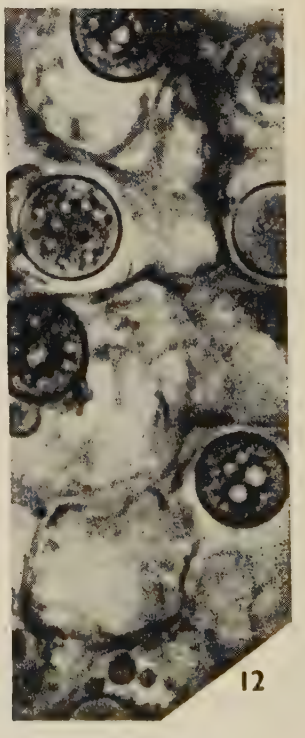
Acropetal serial sections of the apocarpous tomato fruits showed that the pedicel had a complete vascular cylinder (Fig. 13), and vascular strands were given off to the floral organs (Fig. 14), as in the normal case (*see* Esau, 1953, Fig. 18, 1). Growth of the axis evidently continued, however, during the inception of supernumerary carpels, as a result of which a central vascular cylinder was present, in which vascular gaps might occur at the insertion of carpels and lateral axes (Fig. 17). Lateral axes were siphonostelic and were axillary to a carpel (Figs. 17-19). At the point of bifurcation of the fruit axis the vascular tissue also divided, and two vascular cylinders formed (Figs. 20-21). This agrees with the observations of Caldwell (1930) on the vascular configuration of a case of dichotomy in the vegetative axis of a tomato plant. Finally, two separate siphonostelic axes were formed, each of which bore carpels around it (Fig. 22).

Serial sections of half of a partially syncarpous fruit confirmed the presence of lateral, branched axes within the pericarp, cut in various planes. Axes were often cut almost longitudinally at the apex, and more transversely further back (Fig. 25). Branching of the axes was clearly shown (Figs. 23-28). The formation of a second pericarp within the first also occurred, presumably by union of some of the carpels of the lateral axis, and thus the axis might be enclosed within two pericarps (Figs. 23-28). Emergence of the axis through the pericarp was also demonstrated.

In the fruit illustrated in Figs. 13-22, the vascular tissue was apparently quite normal up to the level of insertion of the perianth and androecium, that is, at the levels illustrated in Figs. 13 and 14, but on proceeding acropetally the xylem became very disorganized. Xylem elements were frequently cut longitudinally instead of transversely, and phloem and parenchyma cells appeared to be mingled with the xylem. Disorganization of the xylem did not appear to be quite constant, for intermediate sections showing more normal tissue occurred. However, the







FIGS. 8-12.

fact that the conducting tissue was quite normal below the insertion of the carpels lends support to the suggestion that flower formation proceeded quite normally prior to the inception of the carpels. While penetration of the chromacetic solution in which this fruit was fixed was probably poor, it does not seem likely that these observations could be entirely accounted for by bad fixation.

The free carpels and the outer part of the axis consisted of large-celled, open tissue with a rather crushed appearance (Fig. 9). The cells of the pith more closely resembled normal parenchyma, and in many sections a band of this tissue formed a sort of inner cortex. All tissues of the apocarpous fruits sectioned, however, with the exception of apices, were abnormal in appearance (compare Figs. 8 and 9). In the apocarpous fruits the nuclei were elongated structures applied to the side of the cell, and appeared unhealthy. Those of the partially syncarpous fruit sectioned were more normal, especially near the base of the fruit. However, this may have been partly due to a difference in fixation.

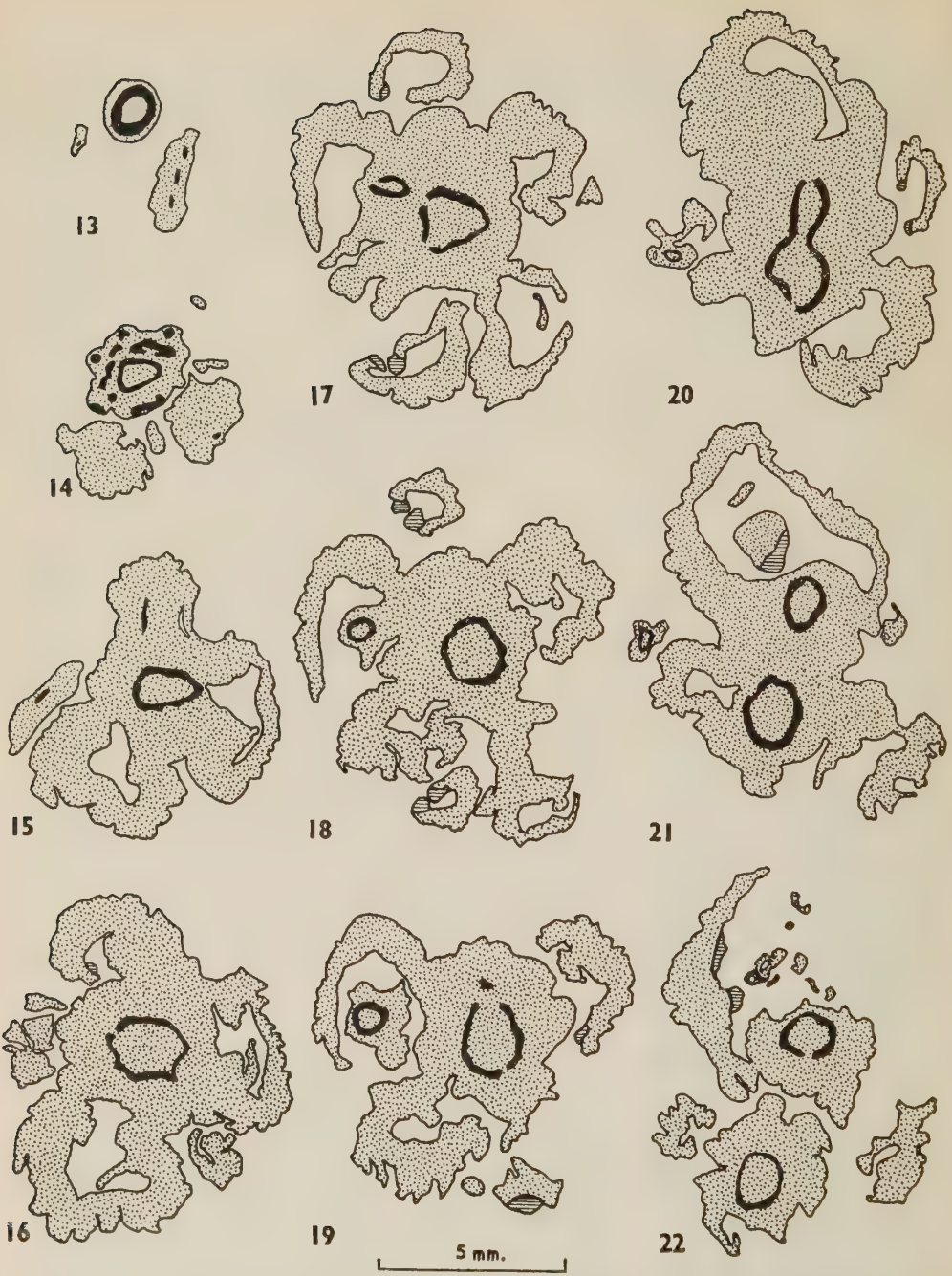
Enations were frequently observed in sections of the apocarpous fruits, usually on the adaxial face of free carpels (Figs. 9, 13-22). In sections of the partially syncarpous fruit only one incipient enation was observed. The cells of these enations contained numerous inclusions which will be described below. It is of interest to note that the enations occupied the positions of the marginal and adaxial meristems of the young carpels near the apex, although this may not be significant. (Compare Fig. 3 with Figs. 9 and 13-22).

Intracellular Inclusions—The enations seemed to have been formed by localized cell division, and were bounded by more darkly-staining seriate cells. Numerous inclusions varying considerably in size were present in the cells of these enations (Fig. 10); small inclusions also occurred in the parenchymatous cells of the pith and inner cortex of the fruit axis, where they were particularly conspicuous in the vascular gaps. Very small inclusions were present in the vascular tissue. Atypical inclusions were very rarely observed near the apical meristem; when present they were very small and very few in number. More than one inclusion was often present in each cell. Inclusions were observed in tissues fixed both in chromacetic and formalin-acetic-alcohol fixatives.

The inclusions were yellowish in colour, and appeared to possess a definite bounding wall, although this could have been an artefact due to fixation. The majority were circular in transverse section. The larger inclusions usually contained numerous refractive granules, but occasionally no free granules were evident; sometimes the contents consisted of one or more globular bodies which might or might not contain a single granule (Figs. 10-12). The granules, like the inclusions themselves, varied considerably in size. Smaller inclusions, including most of those present in parts of the fruit other than the carpellary enations, were usually devoid of granules.

The granules within the inclusions, and also the single granules contained in the globular bodies, were frequently observed to be in rapid movement, which resembled and may well have been identical with Brownian movement. Agitation of the granules was still very marked in sections which had been mounted in canada balsam for over a year. Movement was not manifest in the case of every inclusion, but it has been observed

Figs. 8-12 — Fig. 8. T.s. small normal tomato fruit, showing the two locules, placental tissue and seeds. Vascular tissue darkly stained. × 7·4. Fig. 9. T.s. apocarpous abnormal fruit, showing the siphonostelic axis surrounded by free carpels. On the two free carpels on the right adaxial and marginal enations containing cell inclusions are present. × 7·4. Fig. 10. Marginal enation on a free carpel, from a t.s. of an apocarpous abnormal fruit. Numerous intra-cellular inclusions of various sizes and degrees of morphological complexity are present. × 138. Fig. 11. Inclusions in the cells of a carpellary enation, showing the granular and globular material within them. × 920. Fig. 12. Inclusions in the cells of a carpellary enation, as above. × 1290.



FIGS. 13-22 — (Vascular tissue black; enations cross-hatched). Acropetal serial t.s. of an apocarpous abnormal fruit. Fig. 13. Pedicel of the fruit with the basal part of a carpel inserted at a higher level. Fig. 14. Vascular traces which supply the other floral parts; axial siphonostele continuing. Figs. 15, 16. Axial siphonostele continuing; a vascular gap is present in Fig. 16. Carpels are present, laterally fused with the axis, enclosing a central space. Figs. 17-19. A siphonostelic lateral axis is inserted on the axis axillary to a carpel. Enations containing numerous cell inclusions are present marginally on several free carpels. Figs. 20-22. Bifurcation of the fruit axis, resulting in the formation of two separate, siphonostelic, carpel-bearing axes.

in many examples taken from a considerable number of slides.

The tissues of the carpellary enations stained brownish-yellow with iodine and a reddish colour with Millon's reagent, thus showing a positive reaction to tests for protein. This reaction, however, was not restricted to the cell inclusions, and indeed they themselves did not appear to show very much response to the tests. The outer, seriate cells of the enations, which stained more darkly with haematoxylin (Fig. 10), showed the greatest response. Tests on fresh material would probably give a more reliable indication of the chemical nature of the inclusions, since fixation may well have had an effect. The carpellary enations, however, were the only regions of the fruits which gave any reaction with Millon's reagent.

In sections stained with Feulgen's reagent some of the smaller, less elaborate bodies took up the stain to some extent, but in the absence of recognizable normal nuclei for comparison this cannot be regarded as proof of the presence of deoxyribonucleic acid. The cuticle of the epidermal cells and the lignified tissues present in the sections stained more deeply than the inclusions; Hillary (1939) has already noted that lignin, suberin, cutin, and various polysaccharides may stain with Feulgen's reagent.

From a comparison of published photographs (e.g. Goldstein, 1926, Fig. 3) and descriptive data with these observations, it appears possible that these inclusions may be virus X-bodies.

Discussion

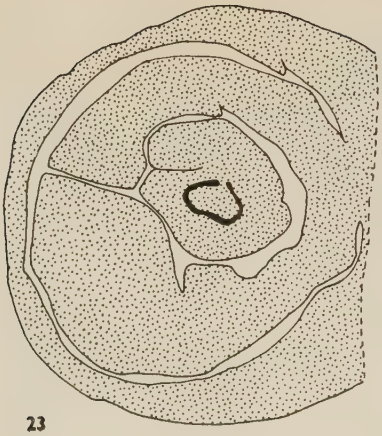
From the foregoing account of the morphology and anatomy of these anomalous tomato fruits two main subjects for discussion emerge, namely the cause of the abnormality and the ontogenetic development of the fruits. Unfortunately, in the absence of fresh material and of fruits in an early stage of development, any such discussion must necessarily remain rather tentative and inconclusive.

The Formation and Development of the Fruits — A morphological appraisal of the available material, in conjunction with the anatomical observations, suggests that the inception of floral organs proceeded normally prior to the inception of the carpels. Perianth and staminal members appeared normal and had undergone normal cohesion and adhesion; moreover, no elongation of the axis, such as characterizes the gynoecium, had occurred between the successive whorls of these members. The factor responsible for the abnormality, therefore, presumably did not exert its effect upon the floral apex until the phase of carpel formation.

As a tentative hypothesis, therefore, it may be suggested that the abnormal fruits could have resulted from the retention by the floral apex, while in the carpellary phase of development, of the capacity for branched axial growth and the continued production of carpels. This type of growth would result in a departure from the normal syncarpous development. Moreover, biochemical conditions in the apex must have remained relatively constant, since nor eversions to the antecedent androecial or perianth phases were observed.

Thompson (1943-44) considers that the number of floral parts of any category is dependent upon the period during which no further alteration in the physiological state of the apex has occurred. The present hypothesis is in substantial accord with this interpretation of normal meristic variation of flowers with high numbers of floral parts. Support for this view is also forthcoming from observations on the lateral axes of the tomato fruits, which were formed during the extended carpellary phase of the parent axis, and apparently commenced their own activity by the inception of carpels.

Perhaps the greatest morphological interest of these specimens lies in the expression of indeterminate growth by a normally determinate organ. Continued growth of the floral axis has been observed on previous occasions, and indeed appears to be a not infrequent occurrence in the Solanaceae. Murray (1945) records the continued meristematic growth of the floral apex of the egg-plant, with the formation of a second set of carpels, and states that this has been observed also in pepper and tomato. In the carob tree (Leguminosae), the floral apex



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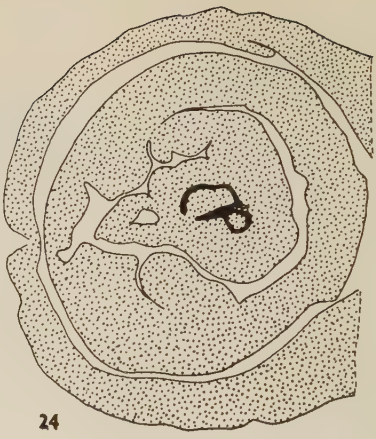

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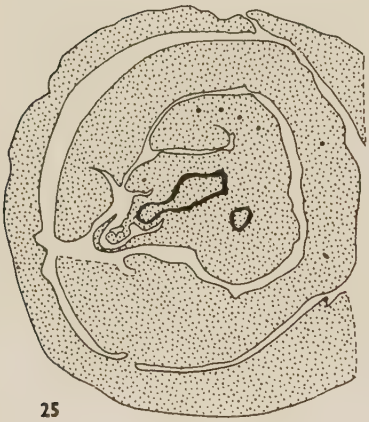

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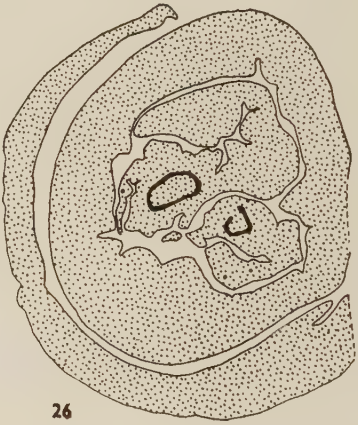

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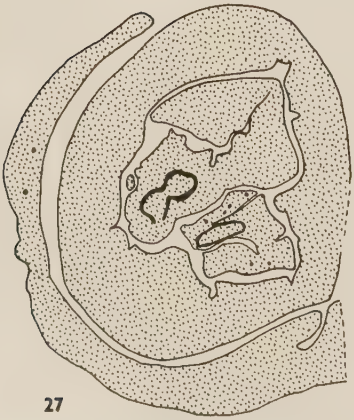

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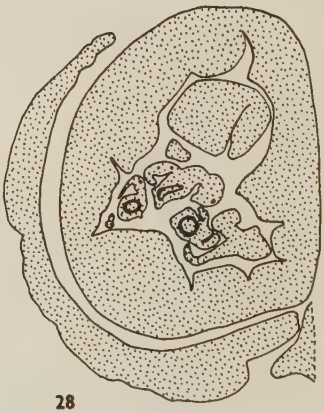

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5 mm.

FIGS. 23-28.

occasionally continues growth as a foliar bud (Thompson, 1943-44). In the present case, however, it is noteworthy that all the fruits of one plant showed indeterminate growth, that is, it was not an isolated phenomenon concerning only a few flowers.

The problem arises as to why the fruits developed at all after the inception of carpels. Normally, fruits develop as a result of fertilization of the ovules, but in these specimens it appears that no fertilization occurred, and indeed that ovule formation never took place. Thus the normal climax of flowering, the formation and fertilization of ovules, was evidently never attained. It appears, therefore, that some other source of auxin or other stimulus must have been available to these fruits, but the grower states that the plants were not given hormone treatment. The hormone balance of the developing fruits must, therefore, have been considerably disturbed, and it seems probable that the production in the floral apex of a hormone stimulating fruit development must have been a feature of the abnormal condition.

The Nature of the Causal Agent — The abnormal fruits bear a superficial resemblance to some fasciated fruits caused by a genetic abnormality described by Zielinski (1948), but differ from the latter in that they were entirely seedless and there was evidently no abnormality of floral parts other than the carpels. Illustrations of the fasciated fruits (Zielinski, 1948) show no apices comparable with those described above and no continuation of axial growth. Moreover, the apices of the fruits described here were apparently normal and showed none of the abnormalities of shape or size usually associated with fasciation (Schoute, 1936; White, 1948).

A genetic variation of the tomato which bears a close resemblance to the condition under discussion is that known as the ' wiry ' tomato (Lesley & Lesley, 1928). As in the present case this affected the leaves and fruits of the plant. The leaves of the wiry tomato were filiform in character; those of the plant under investigation were not received for examination but were stated by the grower to possess unusually long leaflet stalks. In the wiry tomato, as in the present case, the ovary was partially apocarpous, with separate, curled styles. Illustrations of the ovary and fruit of the wiry tomato show no evidence of continued axial growth, and ovules are present, although the fruits were entirely seedless. The authors, while recognizing that the plants bear a considerable resemblance to plants suffering from certain virus diseases, conclude that wiry is the result of a gene mutation, ' wiry ' being recessive to the normal.

Although the abnormal fruits described here bear a certain superficial resemblance to the genetic variations of the tomato described by Zielinski (1948) and Lesley & Lesley (1928), it is clear that they are not identical with the fruits described by these authors and indeed must have resulted from still more profound changes in the formative regions. From a comparison of wiry with other tomato mutations Lesley & Lesley (1928) conclude that the changes induced by a gene mutation in the case of wiry are more profound than those induced in any case then known by the addition of a whole extra chromosome. By inference, therefore, it appears that morphological abnormalities resulting from genetic variations of this type in the tomato are usually less profound than those characteristic of the fruits now under

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Figs. 23-28 — (Vascular tissue black; broken line indicates torn or severed tissue). Acropetal serial t.s. of half of a partially syncarpous abnormal fruit. Figs. 23, 24. Internal axis now enclosed within two pericarps. In Fig. 24 unequal bifurcation of the axis is beginning. Figs. 25, 26. Branching of the internal axis results in the formation of two separate, siphonostelic axes. Figs. 27, 28. Bifurcation of the larger of the two axes results in the formation of three internal axes.

consideration. Notwithstanding these considerations it still remains possible that the fruits may be the result of genetic variation.

The general morphological resemblance between the intra-cellular inclusions found in the abnormal fruits and the X-bodies produced in the cells of plants infected with a virus disease raises the possibility that the causal agent might have been a virus. The inclusions described and illustrated above (Figs. 11, 12) appear to have a more distinct structure than the usual virus inclusion, comparing these illustrations with published photographs, but virus inclusions are very variable. The greatest accumulation of the inclusions was found in specific locations, namely in the cells of enations on the adaxial face of free carpels of apocarpous fruits. Enations frequently occur on the leaves of virus-infected plants (Bawden, 1950).

Bawden (1950) states that virus X-bodies give all the normal protein reactions and never stain with Feulgen's reagent. Staining of the inclusions in the carpellary enations with Feulgen's reagent and tests with Millon's reagent and iodine gave rather inconclusive results.

Many virus diseases cause profound morphological changes in various plant organs. Ovarial tissue appears to be frequently affected, and the occurrence of normal leaves or leafy outgrowths in this region is a feature of both big bud disease (Butler & Jones, 1949) and aspermy of the tomato (Wilkinson, 1953). In big bud disease the shoot is dichotomously branched and the only indications of ovules are small hyaline papillae; the occurrence of continued axial growth in the flowers of tomatoes infected with this disease is particularly striking in its similarity to the present case, but in the fruits under investigation there was no indication of the formation of normal vegetative leaves, nor were the sepals affected in the manner characteristic of big bud disease in the tomato (Kunkel, 1954).

If the abnormalities described here were indeed caused by a virus it seems likely that it must be a disease not previously reported for the tomato in this country. However, Bawden (1950) points out that very different symptoms may be produced by the same virus in different plants and also that the presence of a second unrelated virus in the infected plant may exert a profound effect on the symptoms shown.

Harrison (1952) considers that many extreme examples of teratology are due to inadequate transport or distribution of auxins, which will affect normal growth co-ordination. Viruses are considered to interfere with specific growth processes in the plant (Went, 1951), and they may also affect its nutritional state (Wynd, 1943). Either of these conditions might have a profound effect on morphogenetic processes.

It is clear that without a further supply of fresh material of this interesting abnormality no definite conclusion can be reached as to the nature of the causal agent. The similarity between abnormalities resulting from genic mutations and virus infection has been frequently stressed by many workers. Many variations in *Oenothera* and *Cymbalaria*, particularly those involving floral abnormality, are now thought to be due to a virus disease (Chevalier, 1949), and Wilkinson (1953) considers that many unexplained abnormalities in *Lycopersicon* may also prove to be the result of virus infection. Findlay (1951) contends that, because both mutated and virus-infected cells have a different nucleo-protein content from the cell from which they have been derived, the virus-infected cell may indeed be regarded as a mutated cell. Genetic variations and virus infections are, therefore, likely to affect the same biochemical processes in the organism.

Summary

The morphology and anatomy of some abnormal tomato fruits of the variety Sutton's Ailsa Craig have been examined. An interpretation of their formation is offered, and the possible causal factors concerned are discussed.

The fruits were entirely or partially apocarpous, and had apparently been formed by the continued growth of the floral axis and the production of supernumerary carpels. Branching was a frequent

characteristic of the fruit axes, and lateral axes axillary to a carpel also occurred. These lateral axes bore only carpels and were similar to the parent axis. The carpels were of dorsiventral symmetry and arranged spirally on the axis.

The fruit axes were siphonostelic and division of the central stele occurred at the points of axial bifurcation. Apical organization was apparently normal, and there was no evidence of fasciation. On the adaxial faces of free carpels enations sometimes occurred, in the cells of which were numerous inclusions.

The general resemblance of these inclusions to virus X-bodies suggested that the causal factor might be a virus. This possibility and possible genetic causes are fully discussed, and it is concluded that while no definite conclusion can be reached without further material, it is in any case probable that both these agents would affect the same biochemical processes in the plant.

I wish to express my grateful thanks to Professor C. W. Wardlaw, under whose supervision this work was carried out, for his stimulating guidance and advice throughout the investigation; to Mr. W. Ward and Dr. H. R. Fletcher for the abnormal tomato material, and to Dr. J. A. Macdonald, Interim Keeper of the University Botanic Gardens, St. Andrews, for access to normal tomato material. I am grateful to Miss A. Wylie for advice and assistance on the Feulgen test, and to Mr. E. Ashby, Mr. G. Barker and Mr. P. T. Dawes for the photographic illustrations.

Thanks are also due to the University of St. Andrews for the award of a Berry Scholarship, during the tenure of which this work was carried out.

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MORPHOGENESIS IN *SOLANUM TUBEROSUM* L. : EXPERIMENTAL INVESTIGATION OF LEAF DORSIVENTRALITY AND ORIENTATION IN THE JUVENILE SHOOT

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Introduction

The leaf is the principal organ of photosynthesis in vascular plants, and as such considerable importance attaches both to its disposition on the stem in relation to light, and to its structure in relation to the biochemical processes which occur within it. Dorsiventrality, which confers on the *mature* leaf its special physiological properties, is attained by the *primordial* leaf while still enclosed in the apical bud, and it is, therefore, of interest to examine the causes of leaf dorsiventrality and orientation. In the fern *Dryopteris aristata* Wardlaw (1949a), and Cutter (1954) obtained buds at positions which would normally have been occupied by leaves, and from very young emergent leaf primordia when these were isolated by a tangential incision from the apical meristem of the shoot.

In a series of experiments the apical meristem in the juvenile shoot of potato was surgically isolated from laterally adjacent tissue by 4 vertical incisions (Fig. 1a). The first leaf primordium to emerge after the operation occupied the I_1 position on the apical flank, and was separated from the centre of the meristem by one of the incisions. This primordium was frequently of unusual form, being slender, radially symmetrical, and of more limited terminal growth than dorsiventral leaves (Fig. 3).

In further experiments the positions of the isolating incisions were varied, and it was observed that a radially symmetrical primordium emerged at I_1 only when an incision had passed close inside the I_1 position. When the incision was nearer to the summit of the apical meristem, and relatively far from the I_1 position, I_1 developed as a dorsiventral leaf. It

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therefore appears that the dorsiventrality of a potato leaf may be modified, and in this paper experiments are described which throw some light on the causes of dorsiventrality and orientation of the leaves. Some of these experiments have previously been reported briefly (Sussex, 1951; 1954).

Material and Methods

Rapidly-growing juvenile shoots of named varieties of potato were obtained by a method described previously (Sussex, 1955). The shoots, which grew from cylindrical plugs of tuber tissue 15 × 15 mm, were placed in a dark incubator at 25°C. for about 7 days before being used in experimental treatments. Under these conditions the shoots, which were 15-20 mm in height, were etiolated, roots protruded from the basal nodes, and the leaves were simple scales 2-4 mm in length (Fig. 2). The variety Kerr's Pink was used most extensively, but each treatment was also carried out on other varieties. The results were in the main confirmatory, and will be described separately only when they differed from the Kerr's Pink result.

The shoot apex was exposed for operations by removing outer leaves from the terminal bud until the apical meristem was clearly visible as a translucent, glistening yellowish or pale green mound which varied in diameter from 70-200 μ in the different varieties used. Usually only the 2 youngest leaf primordia, here called P_1 and P_2, P_1 being the younger, were left attached, as they did not obscure the apical meristem from view. Removal

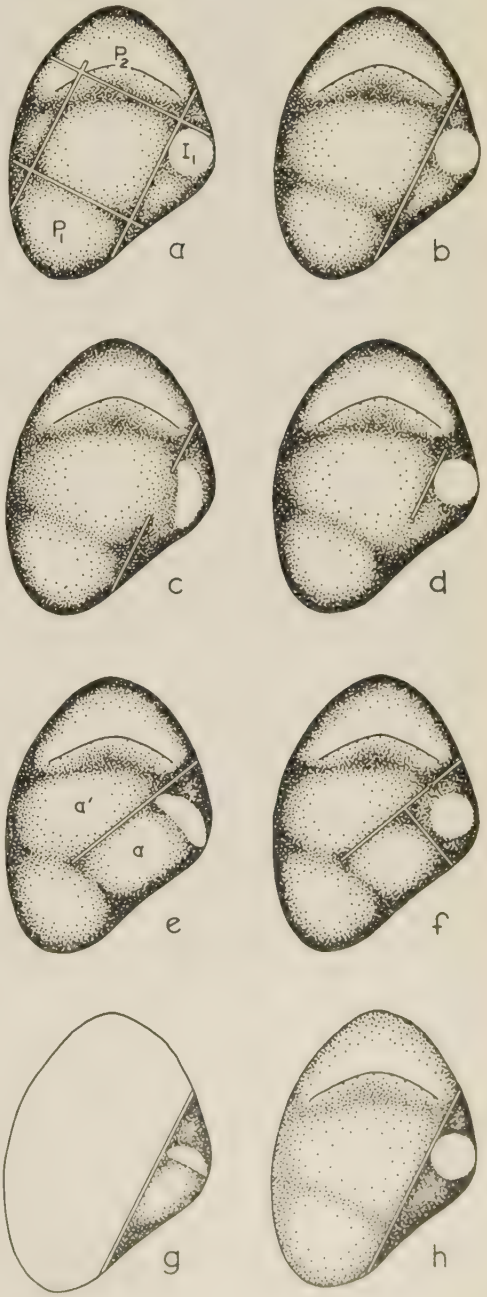


FIG. 1 — Diagrammatic plan of apex showing the position of the various incisions by which the I_1 presumptive position was isolated. P_1 and P_2 are the two youngest leaf primordia present at the time of the operation. I_1, the first leaf to appear after the operation, is drawn to a smaller scale than the other leaves. I_1 is dorsiventral in (c), (e) and (g), and in (e) and (g) is orientated towards the regenerating apex *a*. In (a), (b), (d), (f) and (h) I_1 is centric. In (g) meristematic tissue lying on the distal side of the incision was excised at the commencement of the experiment, in (h) excision of this tissue (lightly stippled) was delayed for about 24 hours. The phyllotactic spiral of the shoot progresses in the anticlockwise direction. × 150.

of leaf primordia and subsequent operations and observations were carried out at a magnification of $\times 100$ using a binocular dissecting microscope. At high magnifications it proved difficult to obtain critical illumination without overheating and consequent drying of the apical tissues. The light was, therefore, passed through a heat absorbing glass filter with which temperatures at the apex were kept below 23°C. Incisions in the shoot apex were made with microscalpels of triangular fragments of razor blades soldered to sewing needles. Because of the small size of the potato apex it was necessary to grind down the back and sides of the microscalpels which otherwise obscured most of the apex during the operation. All incisions were made with the microscalpel held in a screw-driven Leitz micromanipulator, and it has been possible to obtain considerable uniformity in replications of each experimental treatment.

Each operation has usually been repeated at least 15 times, 5-6 replicates being used on each of 3 occasions, and when it was desired to fix material showing intermediate stages of development for microscopical examination additional replicates were used. In treatments in which more than one incision was made in the apex it was usual to allow a recovery period of 1-2 hours after each incision. Otherwise there was a fairly high mortality rate, probably due to the rapid rate of water loss which occurred from incised apices exposed to the air.

The dissection and operation of an apex could be completed in about 5 minutes, after which the shoot was covered with a water-moistened cotton wool cap and placed on filter paper moistened with Knop's solution in a covered glass dish. For the first 12-24 hours after the completion of an operation it was necessary to maintain a high humidity around the apices. Dishes containing experimental shoots were, therefore, left in a cool part of the laboratory for this time, then returned to the incubator and maintained at 25°C. After 3-4 days the cotton wool caps could be removed permanently from the shoots if so desired.

Provided the above precautions are taken the apex of potato is surprisingly resistant to mechanical injury. Mortality resulting from immediate post-operative tissue collapse was less than one per cent of all experimental shoots used in most varieties.

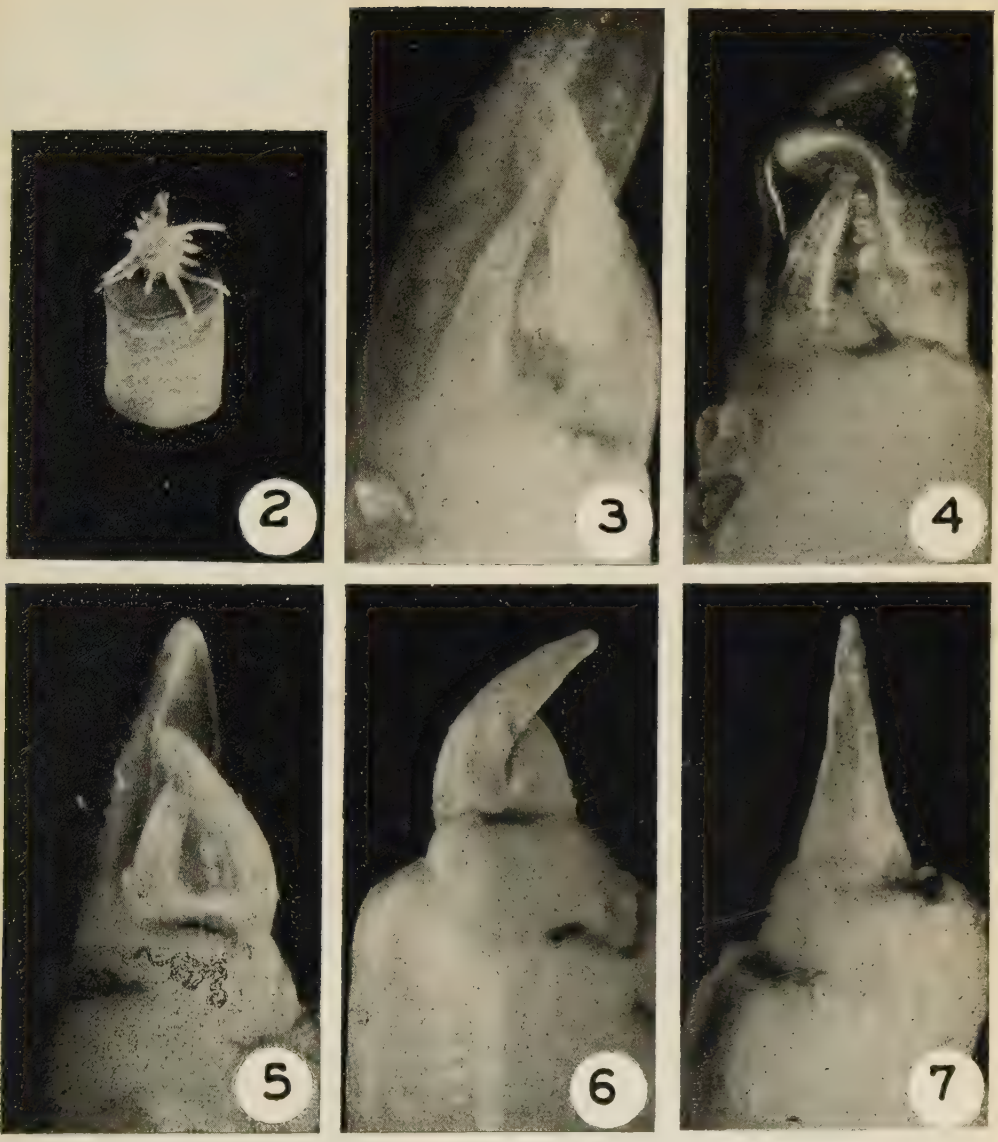
In later stages of growth some shoots succumbed to fungal or bacterial infection, but neither reached serious proportions and no attempt was made to prevent them.

Camera lucida drawings were made during the first 6 hours after an operation to indicate the extent of the incision in each apex. Subsequent observations and drawings were made at intervals of 1-2 days, frequent observation being necessary as the plastochron interval of shoots growing under the experimental conditions was approximately one day.

Leaf Development

The origin of leaves at the margin of the apical meristem in the juvenile shoot of potato and their early ontogeny were described previously (Sussex, 1955); however, certain features which are relevant to the present discussion may be indicated. I_1, the next leaf primordium to appear, emerges on the flank of the apical meristem as a hemispherical mound at the summit of a foliar buttress which begins to enlarge before the leaf emerges. The primordium encroaches laterally and distally over the adjacent apical surface, the adaxial surface then becomes flattened, and the leaf becomes dorsiventrally symmetrical before the end of its first plastochron of growth. Successive leaves arise with an angular divergence of approximately 138°, and lie above and between 2 leaves which are respectively 2 and 3 plastochrons older. Phyllotaxis is $\frac{2}{5}$ or $2+3$.

The 2-layered tunica and outer 2 layers of the 3-layered corpus of the apical meristem contribute cells to the leaf primordium. When a leaf primordium first becomes emergent and is still circular in section there are equal numbers of cells along its tangential and radial axes, but in an older primordium in which dorsiventrality is becoming evident, there are more cells which were derived from



FIGS. 2-7 — Fig. 2. An etiolated juvenile shoot of potato, var. Arran Banner, grown in darkness for 7 days at 25°C. × 1. Fig. 3. Centric I_1 produced after operation (*a*) Fig. 1. Fig. 4. Centric I_1 produced after operation (*f*) Fig. 1. The two incisions in the apex are visible to the right of I_1. Fig. 5. Centric I_1 produced after operation (*b*) Fig. 1. Fig. 6. Dorsiventral I_1 orientated towards the regenerating apex produced after operation (*g*) Fig. 1. Fig. 7. Enlarged centric I_1 produced after operation (*h*) Fig. 1. Figs. 3-7 all × 40. In Fig. 3 the shoot is of var. Stomont Dawn, in Fig. 4 Ulster Chieftain, and in Figs. 5-7 Kerr's Pink. In Figs. 3-6 the phyllotatic spiral progresses in the clockwise direction, and I_1 is on the left front flank of the apex. The shoots in Figs. 5-7 were photographed 5 days after commencement of the experiment.

the corpus lying along the tangential axis than along the radial axis. Thus the initial dorsiventrality of a leaf primordium is the result of unequal rates of growth along the tangential and radial axes.

As the leaf elongates further, vacuolation commences in epidermal cells on both faces. In abaxial cells vacuolation is earlier in its inception, and spreads through the leaf tissues more rapidly than vacuolation in adaxial cells, so that from an early age the 2 faces of the leaf differ in their pattern of development. Between the vacuolating areas an arc of nonvacuolated residual meristem persists, extended laterally across the leaf. From the residual meristem procambium and lateral submarginal initials are differentiated. It is by continued lateral growth of the marginal meristem that the lamina is formed and the dorsiventrality of the leaf further emphasized. The leaves of potato shoots which have grown in darkness are usually simple in outline, lateral leaflets are only rarely developed, and there is no internal differentiation into palisade and spongy mesophyll (Fig. 8).

The position occupied by a newly-emergent leaf primordium on the flank of the shoot apex is one of considerable histological diversity. Distally lie unvacuolated cells of the apical meristem, proximally and laterally epidermal and cortical cells are rapidly vacuolating, and it has been suggested by Wardlaw (1949a) that diversity in contiguous tissues may contribute to dorsiventrality of the leaf, either through the unequal rates of growth which prevail above and below the primordial locus, or through unequal rates of movement of metabolites and growth regulating substances to each face of the primordium. As primordial growth rate is dependent on metabolic supply it may be difficult in practice to distinguish between these in morphogenesis, but it is possible to suggest 3 different regions of the shoot from which substances which might be expected to affect the growth of a leaf primordium may proceed; the subjacent part of the shoot, from which nutrients move acropetally towards the apex; the apical meristem, from which substances, possibly of a hormonal nature, may move towards the young leaf; and the adjacent leaf primordia, which were shown by Wardlaw (1949c) to modify the rate of growth of an intervening younger primordium in *Dryopteris*.

In the preliminary experiments described previously the incisions were deep, and may have modified translocation of substances from more than one of the above regions. Operations were, therefore, planned by which it was hoped to prevent translocation from any one of the 3 regions to an emergent leaf or a presumptive leaf position.

Experimental Results

Isolation of I_1 from Subjacent Tissues — This treatment consists of undercutting the presumptive position of a leaf, and has previously been carried out by Snow & Snow (1947) on *Lupinus*, and by Wardlaw (1949c) on *Dryopteris*. The results obtained with these species agree, for, except when an incision damaged the leaf-forming area itself, a dorsiventral leaf was formed in its expected position above the incision.

The I_1 presumptive position of the potato apex was undercut by a horizontal incision which was placed just above the level of the axil of P_2. The incision

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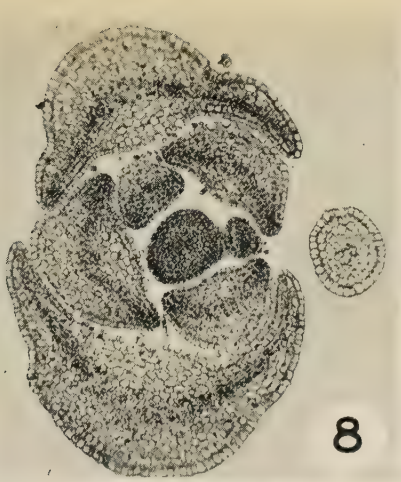
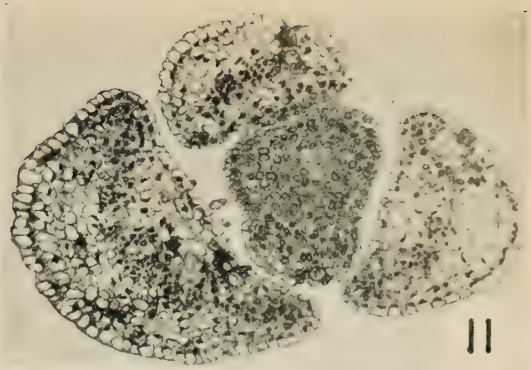
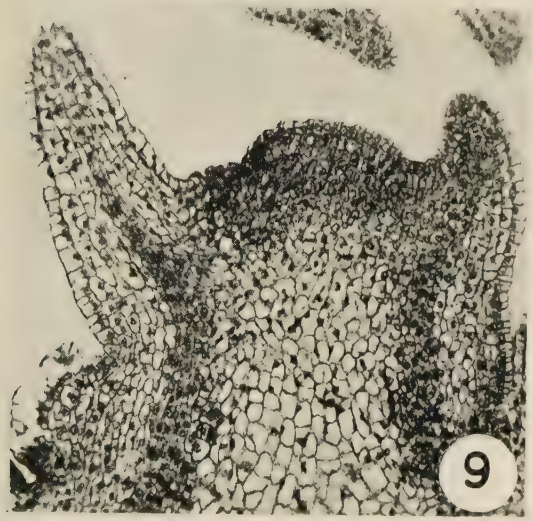
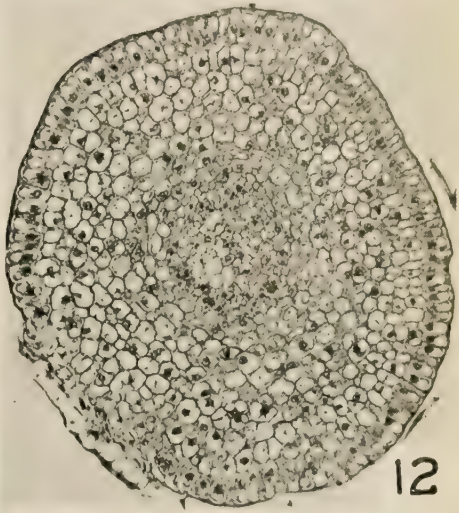
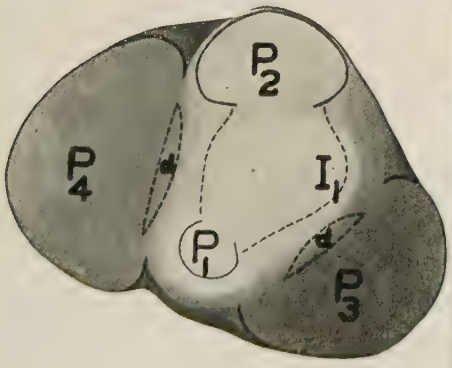
Figs. 8-13 — Fig. 8. T.s. shoot showing a centric protostelic I_1 on the right. P_1 (top) and P_2 (bottom) at the time of the operation are now the large dorsiventral leaves enclosing the apical bud in which 5 new leaf primordia have developed. × 75. Fig. 9. L.s. shoot showing a centric protostelic I_1 on the left flank of the apex. The incision, which has become distended during growth, is the dark area in the apex just above I_1. Fig. 10. Diagram of the shoot apex seen in plan showing the relationship between the apical meristem and the 4 youngest leaf primordia. The margin of the apical meristem is indicated by the broken line. In the axils of P_3 and P_4 are detached meristems (*d*) from which the axillary buds will develop. Successive layers in the diagram, indicated by differences of tone, are 6 μ in depth. Fig. 11. T.s. bud in Fig. 6. I_1 is the large dorsiventral leaf to the left of the apex. Fig. 12. T.s. immature enlarged centric organ. The vascular tissue is siphonostelic and surrounds the pith. Fig. 13. T.s. mature enlarged centric organ. Figs. 9-13 all × 150. Figs. 8, 11 and 13 are of var. Kerr's Pink, Fig. 9 Arran Banner, and Fig. 12 Arran Peak.

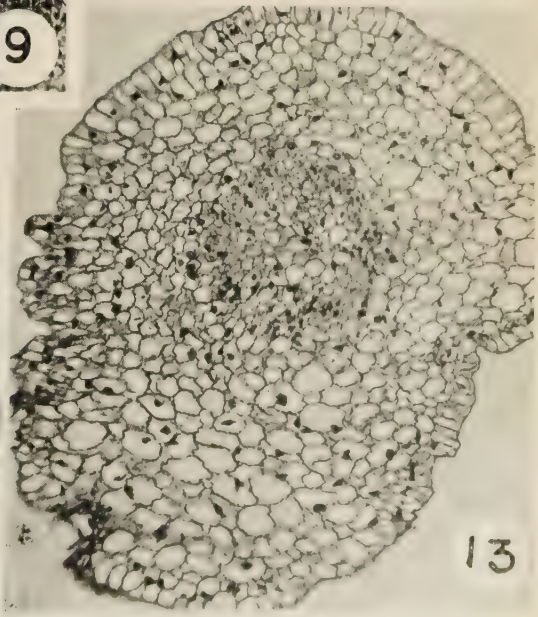












Figs. 8-13.

extended laterally between P_1 and P_2 and inwards almost to the centre of the apex. Because of the translucence of the meristematic tissue the limits of each incision in the shoot apex could be accurately defined. A thin piece of mica was pushed into the incision, and this prevented regrafting of the cut surfaces.

In all those shoots in which the I_1 position was not damaged during the operation, a dorsiventral leaf emerged above the incision. The growth rate of the primordium was initially somewhat less than normal, but at maturity the I_1 leaf was not recognizably smaller than adjacent leaves. Apparently, when acropetal transport of nutrients into a leaf is prevented, the leaf suffers few adverse effects. The nutrients required can be supplied through deeper layers of the shoot at a rate which is adequate to support an approximately normal rate of growth. Dorsiventrality is not modified.

The incision which undercut the I_1 position undercut also approximately one-third of the apical meristem, but during subsequent growth of the shoot the incision remained substantially the same size as when it was made, although the diameter of the shoot increased considerably. In such shoots sectioned for microscopical examination the incision did not approach the centre of the pith but was restricted to a small superficial sector of the stem, and in some the underlying vascular tissue showed no evidence of disruption. This phenomenon has been observed regularly in treatments involving small incisions in the shoot apex. The cessation or decrease of growth in tissues adjacent to the incision is evidently compensated by an increase in the amount of growth in undamaged parts of the shoot apex, and a shoot of nearly normal morphology results.

The relative diminution in size of the incision during the later stages of shoot growth may mean that its effectiveness in preventing acropetal translocation into the I_1 position is gradually diminished. In the present experiment I_1 emerged and became recognizably dorsiventral in 24-48 hours and before there was any considerable growth of the shoot. During the initial phases of growth of I_1, therefore, the incision probably did isolate it from the subjacent tissue as was intended.

Isolation of I_1 from the Shoot Apex — Because of the failure of the preceding treatment to modify leaf shape, it appeared that centric primordia may have developed through separation of the I_1 position from the other meristematic parts of the shoot apex.

The I_1 position was, therefore, isolated from the remainder of the apical meristem and from leaf primordia P_1 and P_2 by a single vertical incision which was sufficiently deep to incise the sloping apical flanks (Fig. 1b).

The incision, which penetrated only the uppermost 6-8 layers of cells in the shoot apex, separated the 5 cell layers of the apical meristem in the I_1 position from those in the distal part of the shoot apex, but tissue continuity in the differentiating subapical region was not disrupted. The isolated flank of the apex was a narrow, tangentially elongated strip of meristematic tissue. The presumptive position of I_1 was not at its centre, but lay closer to the P_2 end (Fig. 1b). Following this operation I_1 emerged on the isolated flank in its expected position, and in 60 isolations I_1 was centric in shape (Fig. 5). In 12 others I_1 developed as a dorsiventral leaf which was usually found to have emerged some distance away from the incision.

The emergence of I_1 was usually delayed for approximately one plastochron, I_1 emerging at about the same time as I_2. I_1 first became evident as a hemispherical mound which was smaller in diameter than normal leaf primordia of comparable age, and remained circular in section throughout its growth. At maturity it was shorter than adjacent dorsiventral leaves.

For each variety of potato it was necessary to determine the exact position in which to make the incision, for in shoots in which the incision had passed relatively far inside the I_1 position a dorsiventral leaf was formed on the isolated apical flank, and if the incision had passed through the tissues of the primordial locus itself no organ was formed, growth being restricted to the

formation of a slight swelling on either side of the incision. In the varieties Kerr's Pink and Ulster Chieftain an incision extending across the apical flank from the margin of P_1 to the margin of P_2 was effective in producing centric I_1 organs.

In var. Majestic, in which the shoot apex is small and the young leaves subtend a wide angle (Sussex, 1955), it was necessary to make the incision just inside the margin of P_2, and in the vars. Stormont Dawn, Arran Peak and Arran Banner, in which the apex is large and newly emergent leaves subtend a narrower angle, the incision was made to pass just outside the margins of P_1 and P_2.

The presumptive position of I_1 has been isolated from the remainder of the apical meristem in plants growing in darkness and in light. Results from both groups are substantially similar, I_1 usually developing as a centric organ. The presumptive positions of I_2 and I_3, the 2 leaf primordia which will emerge next after I_1, have been similarly isolated on 11 occasions. On 9 occasions small centric primordia emerged at these positions in the normal temporal sequence. When the emergent leaf primordia P_2 and P_3 were isolated from the apical meristem by an incision along the axil they continued to grow as dorsiventral leaves. When this operation was performed on newly-emergent P_1 primordia the results were variable. A few of the isolated primordia did not develop further, others became normal dorsiventral leaves, and some developed as centric organs similar to the centric I_1 organs.

In the preceding experiment, the I_1 position was isolated by the incision from the remainder of the apical meristem and from the leaf primordia P_1 and P_2. It is desirable to separate effects of the leaf primordia from those which may be due to the presence of the apical meristem itself. The I_1 position was, therefore, isolated in various ways from either of these regions. Small incisions were made in each flank of the apex to isolate the I_1 position laterally from P_1 and P_2, but the incisions did not meet and so did not disrupt the direct continuity of tissues between the I_1 position and the apical meristem (Fig. 1c). After this operation I_1 emerged as a dorsiventral leaf outside the 2 incisions, and was orientated towards the centre of the apical meristem. The incisions which isolated the I_1 position from P_1 and P_2 were made in the same plane as the incision of the preceding treatment. It is, however, possible to isolate I_1 laterally by 2 parallel incisions. After this operation I_1 was dorsiventral and normally orientated, but was usually of rather small size because of the decreased amount of tissue available for leaf formation between the incisions.

When the I_1 position was isolated from distal apical tissue by a small vertical incision which did not incise the apical flanks, I_1 usually developed as a centric organ (Fig. 1d). The length of the incision was found to be of importance in determining the symmetry of the primordium. A very short incision, or a puncture made with a fine needle, was insufficient to modify the growth of the primordium which, after such experiments, developed dorsiventrally. When only slightly longer incisions were made, I_1 was at first almost centric, but the adaxial face was slightly flattened, and from the margins of the primordium a narrow lamina developed rather late in ontogeny.

From consideration of these treatments it appears that the unequal rates of growth along the tangential and radial axes of a newly emergent leaf primordium are established through the activity of the apical meristem. Isolation of a presumptive leaf position from the meristem results in the development of a centric organ in which the growth rates along the tangential and radial axes are equal.

The Orientation of I_1 — The initial orientation of leaf primordia on the stem would seem to be dependent on the position of the shoot apical meristem, for the slow-growing adaxial face of each leaf would be expected to lie in a plane at right angles to the apical meristem. By experimentally altering the positional relationship between the apical meristem and a presumptive leaf position it should be possible to test this postulate.

A suitable experiment consisted of making an incision which bisected the

apical meristem, passing across the apical summit from the margin of P_2 which lies closer to the I_1 position to the midpoint of P_1 (Fig. 1e). A shoot apex was subsequently regenerated from each half of the bisected meristem. The centre of each regenerating apex lay some distance from the incision, so that in each half of the apex there had been a displacement of the centre of activity. The first leaf to emerge on one of the regenerating apices occupied the I_1 position. The I_1 leaf was dorsiventral, was orientated with its adaxial face towards the regenerating apex *a*, and was deflected up to 25° from its expected orientation in an intact shoot. Additional evidence for apical determination of leaf dorsiventrality was obtained by making a second incision at right angles to the incision bisecting the shoot apex (Fig. 1f). The second incision isolated the I_1 position from the regenerating apex *a*, and following this operation I_1 developed as a centric organ (Fig. 4).

Time of Inception of Leaf Dorsiventrality — Because dorsiventrality may be suppressed by appropriate experimental treatment it appears that growth and dorsiventrality of a leaf are not conjunct, but it remained to be established at what stage in the ontogeny of a leaf the dorsiventral growth pattern was determined. To do this it is necessary only to isolate from the apical meristem the presumptive positions of leaf primordia and emergent leaves of different ages. However, so that the results would be comparable, experimental isolations were restricted to the I_1 presumptive position at different stages of development. The shoot apex was first bisected from the margin of P_2 to the midpoint of P_1 (Fig. 1e) and at succeeding times a second incision which isolated I_1 from the regenerating apex *a* was made at right angles to the first incision (Fig. 1f).

In all 10 shoots in which the second incision was made at the same time or soon after the first, I_1 was centric. Even when I_1 had begun to grow and formed a hemispherical mound on the apical flank, it usually developed as a centric organ when isolated from the regenerating meristem *a* by the second incision. It was only when the second incision was not made until 24-36 hours after the first, and I_1 was already emergent and recognizably dorsiventral, that isolation from the meristem failed to modify its shape. Of 18 such isolations I_1 developed as a dorsiventral leaf in all but 2 shoots, in which it was centric. Once dorsiventrality has been determined in a leaf it does not appear to be readily reversible.

Alternate Modes of Development of I_1 — Alteration of the symmetry of a leaf primordium raises the question of homology between dorsiventral leaves, centric organs and shoots. Wardlaw (1949a) obtained buds in presumptive leaf positions which had been surgically isolated from the apical meristem of *Dryopteris*, and from this and other observations (1950) concludes that leaves and shoots in this fern are initially homologous, their subsequent divergent development resulting from the different conditions which prevail in adjacent tissues during their growth. The centric organs of potato underwent only a limited amount of terminal growth and bore no lateral appendages, their resemblance to shoots being confined to their radial symmetry.

The isolated flanks of the apex from which centric organs developed were of small size, and it has been shown that other small isolated panels of potato apical tissue regenerate as shoots only if the remainder of the shoot apex is excised (Sussex, 1952). If these other tissues are not excised the isolated panel fails to regenerate, and the failure was attributed to unequal competition for nutrients. It seemed possible that competition of a similar nature may have prevented regeneration of the isolated I_1 position as a shoot. If this were so, removal of all other meristematic tissue may allow I_1 to develop as a shoot.

The I_1 position was, therefore, isolated from the remainder of the apical meristem by an incision extending from the margin of P_1 to the margin of P_2 (Fig. 1b). All the meristematic tissue of the shoot apex which lay to the distal side of the incision, and the leaf primordia P_1 and P_2 were then excised by a single sloping cut (Fig. 1g), and the axillary buds of older

leaves destroyed. Following this operation the isolated apical flank enlarged rapidly, became prominently mounded, and acquired the translucent, glistening appearance characteristic of an actively growing apical meristem. The first leaf to arise on such regenerating apices was dorsiventral, and occupied the I_1 position at one end of the isolated apical flank. This leaf was orientated towards the regenerating shoot apex, and thus faced approximately at right angles to its expected orientation in an intact shoot (Fig. 6). Successive leaves emerged around the regenerating apex in spiral sequence, and a small rapidly-growing bud was formed (Fig. 11). Of 32 isolations 28 developed a shoot apex towards which the dorsiventral I_1 leaf was orientated. In the other 4 a shoot apex did not develop, and I_1 was centric.

The regeneration of a shoot apex and the emergence of I_1 as a dorsiventral leaf on the isolated flank were not the results expected from this treatment, but could be explained by considering the distribution of meristematic activity at the shoot apex. The lateral extent of the apical meristem in the juvenile shoot of potato can be determined according to the characteristic plane of division in cells of the second layer of the tunica (Sussex, 1955). The apical meristem is not symmetrical around the shoot tip, but projects into the axils of P_1 and P_2 and into the I_1 position (Fig. 10). In the experiments in which the I_1 position was isolated from the apical meristem by an incision running from the edge of P_1 to the edge of P_2 a small triangular strip of meristematic cells which lie between the I_1 position and P_1 was included on the isolated flank. When this flank developed in competition with the remainder of the meristem, the meristematic cells on the isolated flank failed to regenerate an apex and I_1 was centric. Excision of the distal part of the shoot apex freed the isolated flank from competition. The meristematic cells which had been isolated with I_1 were able to regenerate a shoot apex, and I_1 emerged as a dorsiventral leaf in its expected position, but was orientated towards the new meristem.

In order to test further the regenerative ability of the I_1 position, it was necessary to isolate it from all the meristematic tissue of the shoot apex. The I_1 flank was, therefore, isolated as previously by a single vertical incision (Fig. 1b) and the shoot was allowed to grow for a further period of about 24 hours. I_1 was then visible as a slight hemispherical swelling, and it was expected that the meristematic cells which had been isolated with I_1 would have begun to differentiate. The distal part of the shoot apex and the leaves P_1 and P_2 were then excised, and axillary buds of older leaves destroyed as before (Fig. 1h). The I_1 flank continued its development freed from the competition of other meristematic tissues of the shoot.

The isolated flank enlarged considerably but a shoot apex was not usually regenerated, either at the I_1 position or in the tissue flanking this position. Instead, I_1 continued its development as a centric organ of limited apical growth, gradually encroaching over the entire surface of the isolated flank (Fig. 7). Of 11 isolations 10 developed in the manner described; the other developed as a shoot apex towards which the dorsiventral I_1 leaf was orientated.

Centric organs obtained in this treatment were considerably larger than those of any of the previous treatments. They had a broad base, and were often taller than dorsiventral leaves of similar age (Figs. 6, 7). The tallest centric organ was 2·3 mm in height.

The Development and Structure of Centric Organs

Centric organs vary greatly in their size and structure. Terminal growth in some is of very limited duration and at maturity these form small peg-like projections from the isolated flank. They lack vascular tissue and cause no disruption in the subjacent vascular cylinder in the stem. Below others which are slightly taller there is a small vascular strand which fades out acropetally in the cortex of the stem. In such strands there is only occasional differentiation of xylem and phloem elements, and above

the point of departure of the strand from the vascular cylinder of the stem there is a very narrow parenchymatous gap.

It is usual for centric organs to grow taller than those just described. During their growth centric organs remain approximately vertical; they may curve slightly towards the centre of the shoot apex, but are not as strongly incurved as dorsiventral leaves. At maturity the 'cortex' is 5-10 cells in width, and is composed of uniformly vacuolated parenchyma cells (Figs. 8, 9). Residual meristem, unlike that in a dorsiventral leaf which extends as a continuous arc to the leaf margins, occurs only in the centre where it forms a radially symmetrical column. Procambial and vascular elements do not reach to the vacuolated tip the organ, but appear some distance below it (Fig. 9). Through most of the length of the centric organ vascular tissue is protostelic. The xylem is poorly developed, usually only 3-4 lignified elements being present at any one level. On the outer margin of the vascular column is a narrow zone of small, densely-staining cells, some of which are further differentiated as sieve tubes. Near the base a short length of the vascular tissue is often siphonostelic. The central pith in this region is only a few cells wide, and lignified cells in the xylem are confined to the abaxial side of the organ. Close to the point of junction of the vascular systems of the centric organ and the stem the siphonostele opens on its upper surface, and at the junction of the two vascular systems there is a narrow parenchymatous gap.

Centric primordia which develop freed from the competition of other meristematic tissue are much more robust (Figs. 12, 13). Close to the vacuolated tip, vascular tissue is protostelic, but below this, and throughout most of the length of the organ, there is a conspicuous siphonostele in which lignified cells occur at several points. Phloem is differentiated on both the inner and outer margins of the cylinder, and the pith is of relatively large diameter.

Axillary buds or bud meristems have not been found in association with centric organs.

Discussion

Although the experiments reported in this paper have revealed phenomena for which adequate explanations cannot yet be offered, the data indicate clearly that the positions at which leaf primordia emerge on the flank of the apical meristem possess potentialities for development other than those which normally become manifest. The dorsiventral mode of growth, which is one of the most distinctive characteristics of the leaves of vascular plants, is only one of several developmental possibilities. The inception of growth and the dorsiventrality of a leaf primordium are not conjunct, and by appropriate experimental treatment dorsiventrality can be suppressed, the primordium developing as a centric organ in which tissues are distributed radially.

The formation of radially symmetrical organs in the place of dorsiventral leaves has been reported on several occasions. Radial organs were observed by Versluys (1921) to have developed in cherry from the remainder of the apical meristem after the formation of floral primordia, and by Lang (1924) in the fern *Osmunda regalis* after destruction of the shoot apex. Snow & Snow (1935) obtained radial organs following bisections of the shoot apex of *Epilobium hirsutum* in which the meristem was split unequally. The smaller side of the apex often failed to regenerate completely; only one or 2 leaf primordia emerged on it, and the last was frequently terminal and radially symmetrical. In other experiments with *Epilobium*, Snow & Snow (1942) found that very young emergent leaf primordia when isolated from the apical meristem often developed radially.

Wardlaw (1945, 1947, 1949b) observed that awl-shaped leaf primordia were developed in *Onoclea sensibilis* as the shoot apex became attenuated, or when young emergent leaves of *Dryopteris* were surgically isolated from the apical meristem. These leaves were radially symmetrical, and in the proximal parts at least the vascular tissue formed a complete cylinder. When the presumptive position of a leaf was isolated from the apical meristem in *Dryopteris*, Wardlaw (1949a)

found that a bud was usually developed in place of the expected leaf. In recent experiments Cutter (1954) isolated young emergent leaf primordia of *Dryopteris* from the apical meristem in such a way that they developed as buds. This change could be effected only in a leaf primordium in which a single apical cell had not become dominant. After an apical cell had attained precedence the isolated leaf primordium would no longer develop as a bud.

In each of the examples cited the change from a dorsiventral to a radial growth pattern in the lateral organs follows the cessation of activity in the shoot apical meristem, or the surgical isolation of presumptive leaf positions from it. Results described in this paper support this conclusion and indicate that the dorsiventrality of the leaves of potato is caused by the activity of the shoot apical meristem.

This conclusion has, however, recently been questioned by Snow & Snow (1954a) who used potato shoots to repeat the writer's experiment of isolating the I_1 position from the apical meristem. In only one instance in their experiments did I_1 develop as a radially symmetrical organ; in all other shoots a dorsiventral leaf was formed at I_1. Some possible causes of the discrepancy between the 2 sets of results have previously been discussed (Sussex, 1954; Snow & Snow, 1954b). Snow & Snow (1954a) observed that small radial organs arose between the isolated I_1 leaf and the incision in potato, and they state that in *Epilobium*, but not in other species, radial organs were developed from the central part of P_1, or the presumptive position of I_1 when these were confined at the sides by incisions which did not interrupt the continuity of tissues between the leaf-forming area and the stem apex. They therefore suggest that a radial leaf may develop from an area which is in some way weakened, or is insufficiently large to give rise to a dorsiventral leaf, and that dorsiventrality is not induced by the stem apex. If this conclusion is valid then intrinsic factors, i.e. in the leaf itself, are operative in determining leaf symmetry.

Experiments described in the present paper would appear to permit a distinction between the opposed hypotheses of intrinsic or extrinsic determination of leaf dorsiventrality, and also to provide an answer to the question of decreased size or weakening of the leaf-forming area as the cause of centric organ formation. Only 3 of the experimental treatments, those shown in Figs. 1b, g and h, in which the size of the isolated apical flank was similar, need be considered. I_1, which would have become a dorsiventral leaf in normal development, developed as a centric organ when isolated from the remainder of the apical meristem. When, after isolation of the I_1 position, the distal meristematic tissue of the shoot apex was excised a shoot was regenerated on the isolated flank, and I_1 developed as a dorsiventral leaf orientated towards the new apex. If excision of the distal meristematic tissue was delayed until I_1 was just emergent, apical regeneration did not occur on the isolated flank and I_1 developed as an enlarged centric organ. In the 2 latter treatments the isolated flank enlarged considerably more than in the first treatment, but the symmetry of I_1 was determined not by the size of the basal tissue from which it arose, but by the presence or absence of a contiguous apical meristem. That is, leaf dorsiventrality was determined by extrinsic factors.

This explanation does not in its present form account for the emergence of radial leaves between 2 lateral incisions in *Epilobium*, but is in conformity with the findings of Wardlaw (1949a) and Cutter (1954), and the radial symmetry of isolated organs in *Dryopteris* and the potato, which are members of 2 widely different taxonomic groups, suggests an essential similarity of underlying processes, involving participation of the apical meristem, in the determination of leaf dorsiventrality.

In contrast to the results obtained by Wardlaw (1949a, 1955) and Cutter (1954) using *Dryopteris*, in no angiosperm has the change to a radial pattern of growth in a leaf been accompanied by continued meristematic activity of its tip to form a shoot. The reasons for the determinate apical growth of centric organs of potato

are not obvious. At the time of isolation of I_1 from the apical meristem this position may already have been determined as a leaf-forming area (Snow & Snow, 1933). Very little is known concerning the nature of the determination process but it may result in the limited apical growth of the organ which subsequntly emerges there. The determinate growth of I_1 is not directly consequent on the small initial size of the isolated apical flank, for smaller panels comprising fewer meristematic cells are able to regenerate as shoots under certain experimental conditions (Sussex, 1952). In treatments in which all the meristematic tissue of the shoot except that on the I_1 flank was excised, a shoot apex did regenerate from the isolated meristematic cells. However, reasons have been given for supposing that this did not constitute development of I_1 as a shoot, but that the shoot apex was regenerated from other meristematic cells which had been isolated with I_1. On no occasion has a shoot apex regenerated from cells which undoubtedly formed part of the I_1 position.

The limited apical growth of centric organs which developed in surgically isolated I_2 and I_3 positions is more difficult to interpret, for at the time of isolation from the apical meristem these positions are probably not determined as leaf-forming areas (Snow & Snow, 1933), and since they lie closer to the centre of the meristem than other apical panels which regenerated as shoots after isolation (Sussex, 1952), it seems unlikely that their cells could already have undergone partial differentiation. Evidently isolation from the apical meristem is insufficient to cause a presumptive leaf position at the potato shoot apex to develop as a bud, but the dorsiventrality of the organ which emerges is suppressed. Centric organs have developmental affinities with shoots as well as with leaves. Like shoots they have a symmetrical terminal meristem which lays down tissues behind it in a radial pattern, but like leaves the growth of the meristem is limited.

It is desirable to examine in more detail the relationship between activity of the apical meristem of the shoot and dorsiventrality of the leaf in normal development. Wardlaw (1949a) considers that a leaf primordium of *Dryopteris* develops under conditions where the rate of growth is greater on the abaxial side and where the adaxial side is probably subject to inhibitive effects proceeding from the apical meristem. Within the apical meristem there is a functional centre which regulates certain morphogenetic processes in the developing shoot (Wardlaw, 1949b). In leptosporangiate ferns this centre is characterized by the presence of a conspicuous apical cell, and in angiosperms frequently by a distinctive group of initial cells. The form and symmetry of the leaves are, however, not determined directly by the centre of the apex, for in both ferns (Wardlaw, 1949b) and angiosperms (Pilkington, 1929; Sussex, 1954) dorsiventral leaves continued to emerge on the flanks of apices the centre of which had been destroyed by puncturing. In other experimental treatments newly-emergent leaf primordia isolated together with a narrow strip of apical tissue became dorsiventral (Snow & Snow, 1942; Sussex, 1952). Thus lateral portions of an incised meristem which are contiguous with the leaf-forming area would appear to be effective in determining dorsiventrality in the emerging primordium. That lateral parts of the meristem determine leaf dorsiventrality is strongly suggested by recent experiments carried out by Wardlaw (1955). He isolated newly-emergent leaf primordia or presumptive leaf positions of *Dryopteris austriaca* by 2 diagonal incisions which did not meet, but left a narrow 'bridge' of tissue connecting the meristem with the primordial site. This operation, which was some what similar to that shown in Fig. 1c, resulted in a proportion of buds developing in the partially isolated positions. Wardlaw concluded that the incisions had interfered with the normal movement of hormones and metabolites, and had, therefore, modified growth relationships in the sector of the apex in which the primordium was situated. This resulted in evening-up of growth rates of the adaxial and abaxial sides of the primordium, which then developed as a shoot.

The mechanism by which the apical meristem causes dorsiventrality in a newly emergent leaf is not known, but the consequences are profound and involve both quantitative differences of growth rates along different axes of the leaf, and qualitative differences in the pattern of differentiation. The simplest explanation, that the apical meristem produces a hormone which partially inhibits the growth of cells on the adaxial face of the leaf, fails to account for the considerable tangential growth and dorsiventral distribution of tissues in the leaf. Since in both *Dryopteris* (Cutter, 1954) and potato, dorsiventrality, once established in the leaf, is irreversible as shown by isolation from the meristem of leaves which are already dorsiventral, it seems more probable that the action of the apical meristem is transitory, and is limited to promoting asymmetrical growth in the initially symmetrical meristem of the leaf during its early divisions. Once this action, which may be hormonal in nature, is accomplished, the subsequent dorsiventral growth and tissue distribution of the leaf would be dependent on continued growth of its own dorsiventral terminal meristem.

The action of the apical meristem would then be comparable to the induction of development in one embryonic tissue region by another in animal embryogeny. In the embryos of certain animals induction may be studied experimentally by grafting tissues into unusual positions (Weiss, 1939), but embryonic tissue grafts in the shoot apex of plants have so far proved unsuccessful (Ball, 1950). It is, however, possible to obtain some information relating to the induction of dorsiventrality in a newly-emergent leaf primordium by operations which cause alterations in the spatial relationships of tissues and organs at the shoot apex.

In those experiments on the potato apex in which the centre of the apical meristem was incised or destroyed, new apical centres were regenerated on the flanks of the apical mound. I_1 emerged as a dorsiventral leaf in its expected position, but faced an abnormally situated apex, and not towards the original centre of the shoot. In one series of experiments the deflection of I_1 from its expected orientation in an intact apex was of the order of 25°, in another series approximately 90°. In these latter leaves the parenchymatous adaxial and abaxial tissues were differentiated from regions which in normal development would have become the meristematic leaf margins, while the regions from which the margins of the leaf were formed would normally have become the 2 nonmeristematic faces. Evidently the I_1 position, which was already determined as a position of limited growth when the operation was performed, lacked the stimulus for dorsiventral development. Dorsiventrality was subsequently induced in it by the action of the abnormally situated regenerating shoot apex, and in the dorsiventral primordium tissue differentiation was related to the new plane of dorsiventrality.

The dorsiventrality of a leaf is the result of a complex set of factors working together during ontogeny. The account given here is undoubtedly oversimplified, and many questions remain unanswered. It is hoped that future work will provide a more complete picture of leaf development and the mechanisms by which it is controlled.

Summary

Anatomical evidence indicates that a leaf primordium emerging on the flank of the shoot apex of potato is initially circular in section, but soon becomes dorsiventral as a result of differences of growth rates along its tangential and radial axes.

Experiments in which incisions were made in the shoot apex demonstrate that the dorsiventral growth pattern of a leaf may be suppressed. Dorsiventrality is determined, not by the subjacent part of the stem, or the adjacent leaf primordia, but by the apical meristem. When a presumptive leaf position is surgically isolated from the apical meristem a centric organ of radial symmetry develops. By altering the spatial relationship between apical meristem and presumptive leaf position the leaf emerges

orientated towards the new shoot apex. By isolating presumptive leaf positions and leaf primordia of different ages it is shown that dorsiventrality is not determined until the leaf has formed a fairly large mound on the apical flank. An isolated leaf position undergoes different types of development if the other meristematic tissue of the shoot is excised at different times after the initial isolation.

The tissues of centric organs are laid down in a radial pattern. The smallest centric organs have no vascular tissue, larger ones have a protostele, and the largest have an extensive siphonostelic vascular system.

The views are advanced that centric organs have developmental affinities with shoots as well as with leaves, and that in normal development the action of the shoot apical meristem in causing leaf dorsiventrality is an example of embryonic induction.

It is a pleasure to express my gratitude to Professor C. W. Wardlaw for his encouragement and guidance of this investigation. I also wish to thank Mr. E. Ashby and Mr. P. T. Dawes for the photographic illustrations.

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CONTRIBUTIONS TO THE EMBRYOLOGY OF *ACROTREMA ARNOTTIANUM*

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Acrotrema Jack., the only herbaceous genus of the Dilleniaceae, consists of nearly ten species most of which occur as endemics in Ceylon. There are only two species outside Ceylon: *A. costatum* Jack. in Malaya and *A. arnottianum* Wight in the Indian Peninsula. The latter is confined only to a small part of the Western Ghats in the Tinnevelly District and in the adjoining Travancore borders.

The South Indian species inhabits the monsoon forests and shows a special preference to road cuttings and steep inclines under dense canopied vegetation. The plants possess a semi-woody root-stock embedded in soil, with a highly abbreviated aerial axis. The plants have a tendency to grow in pure patches, and the conspicuously large leaves spread flat on the soil, so as to form a mat (Fig. 39).

The material for this study was collected from Kallar (in the neighbourhood of Ponmudi), Travancore State, in February 1955.

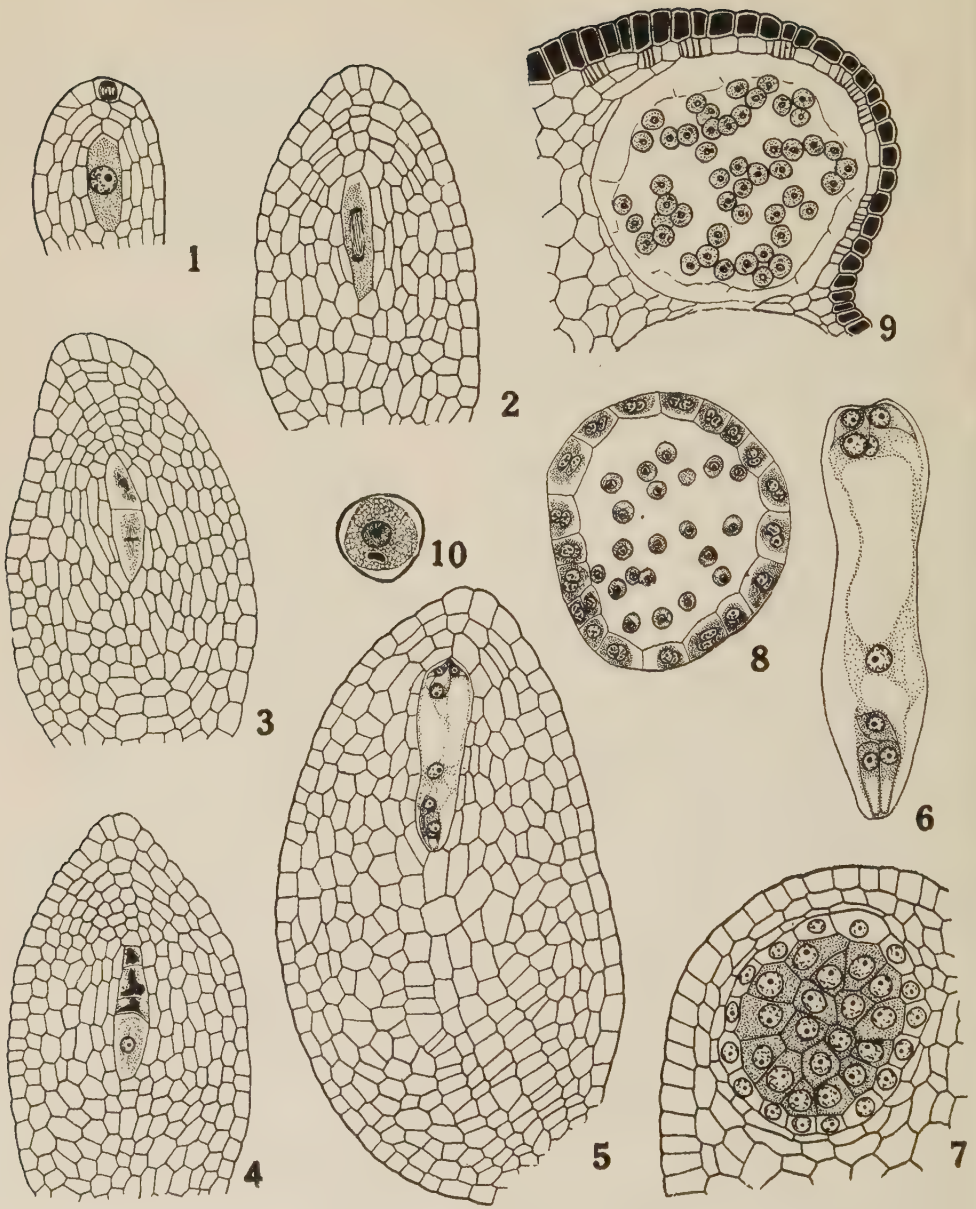
The only published accounts on the embryology of the Dilleniaceae (*sensu* Gilg & Werdermann, 1925) is that of Paetow (1931) on *Wormia suffruticosa* and of Schnarf (1924) on *Hibbertia dentata*. Their observations will be taken up for discussion in subsequent pages.

Observations

Ovule — The gynoecium is syncarpous at the base and apocarpous towards the apical half. The ovules are arranged in two longitudinal series along the placenta in each carpel. To begin with, the ovule primordium arises in the form of a stub-like projection, its apex pointing away from the placenta. The archesporial cell becomes differentiated in the hypodermal layer of the primordium, and this is soon followed by the origin of inner and outer integuments. Hand in hand with these changes, the ovule as a whole undergoes a curvature of more than 90 degrees (Fig. 24). The integuments envelop the nucellus as early as the megaspore mother cell attains the stage of reduction divisions, and by this time the ovule becomes completely anatropous (Fig. 25). At this stage a procambial conducting strand can be traced up to the basal end of the nucellus. The outer integument not only grows at a faster rate than the inner, but also extends beyond and arches over the inner integument (Figs. 25, 26). It is significant to note that the exostome and endostome are not disposed along the same axis, and that in the micropylar part there is always a small space between the outer and inner integuments (Figs. 26, 27).

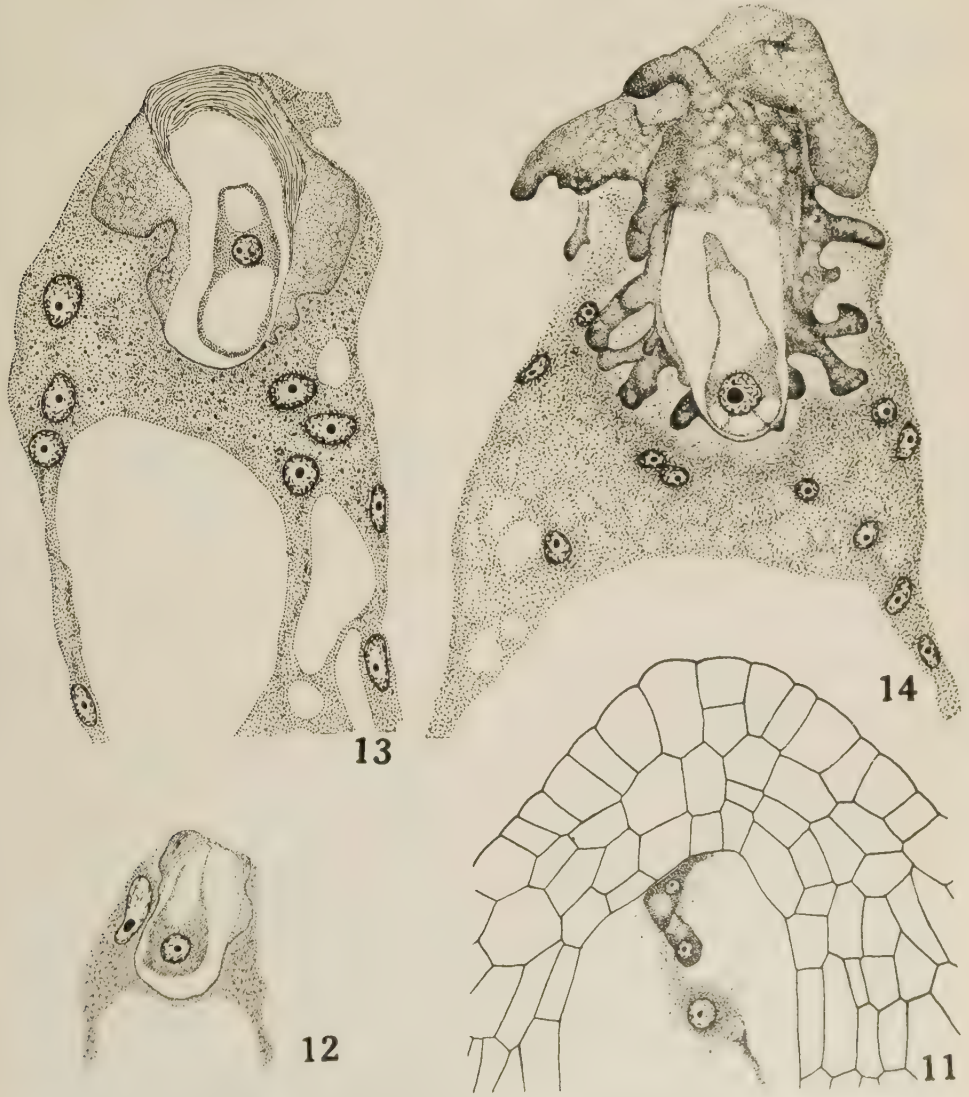
Female Gametophyte — The hypodermal archesporial cell cuts off a primary parietal cell and a sporogenous cell. The former undergoes one or two periclinal divisions thereby pushing the sporogenous cell deeper in the nucellus. Some increase in the parietal tissue is also brought about by one or two periclinal divisions of the nucellar epidermal cells (Fig. 1). The maximum development of the parietal tissue (about eight layers), due to the combined activity of the primary parietal cell and the nucellar epidermis, may be seen at the megaspore tetrad stage (Fig. 4). Much of this tissue becomes disorganized during the linear enlargement of the embryo sac, and at the time of fertilization only two or three layers of parietal cells may be seen between the micropylar end of the embryo sac and the nucellar epidermis (Fig. 5).

The megaspore mother cell undergoes considerable elongation along the longitudinal axis of the nucellus before entering



FIGS. 1-10 — Fig. 1. Nucellus with megaspore mother cell. × 435. Fig. 2. Megaspore mother cell in Meiosis I. × 435. Fig. 3. Dyad cells in division. × 435. Fig. 4. Linear tetrad. × 435. Fig. 5. Nucellus showing chalazal meristem and mature embryo sac. × 435. Fig. 6. Embryo sac before fusion of polar nuclei. × 870. Fig. 7. T.s. young anther loculus. × 390. Fig. 8. Uninuleate microspores surrounded by the binucleate tapetum. × 390. Fig. 9. T.s. mature anther loculus. × 390. Fig. 10. Mature pollen grain. × 870.

the meiotic divisions. As a result of the meiotic divisions (Figs. 2, 3) a linear tetrad of megaspores is organized (Fig. 4). Hand in hand with the further development of the female gametophyte, the nucellar cells at the chalaza become meristematic (Fig. 5) and this activity continues, although at a retarded rate, even after fertilization. The continued activity of the chalazal meristem on the sector away from the raphe is largely responsible for the amphitropous condition of the ovule and seed during post-fertilization development (Figs. 27, 28). The chalazal megaspore, by three successive divisions, builds up an 8-nucleate embryo sac. The antipodals are organized as cells; two of them are elongate with



FIGS. 11-14 — Fig. 11. Micropylar part of the nucellus and embryo sac soon after fertilization. Fig. 12. Swelling of the zygotic membrane. Figs. 13, 14. Successive stages in the development of the zygotic mantle. All Figs. × 870.

conspicuous basal vacuoles and lie juxtaposed; the third antipodal cell is generally situated somewhat anterior to the other two and does not exhibit any conspicuous vacuolation. According to Schnarf (1924), the antipodals degenerate very early in *Hibbertia dentata*. The synergids possess an inverted pyriform shape (Figs. 5, 6). The polar nuclei fuse at a later stage to form the secondary embryo sac nucleus (Fig. 5).

Microsporogenesis—The youngest anther that was available for examination showed the epidermis, two-wall layers, and the tapetum, followed by a mass of microspore mother cells (Fig. 7). The tapetal cells become binucleate when the microspore mother cells undergo the prophasic changes leading to the meiotic divisions, and its further behaviour conforms to the secretory type (Fig. 8).

It is interesting to note in this connection that Paetow (1931) has described a periplasmodial organization of the tapetum in *Wormia*. In view of the lack of convincing illustrations to support such an interpretation and also in view of our own oservations to the contrary in *Acrotrema*, the situation in *Wormia* needs confirmation. A second point mentioned by Paetow concerns the breaking down of the tapetal cell walls along the sides of the locule nearer to the axis of the anther (*see* Paetow's Fig. 12). We are unable to confirm this phenomenon in *Acrotrema*. It is true that sometimes the tapetal layer shows an apparent separation from the anther wall. This, we consider, is due to artifacts brought about by excessive shrinkage or by improper methods of handling the material while sectioning. In *Acrotrema*, the peak activity of the tapetum is overbefore the microspores undergo the first division, and it is completely disorganized in the mature anther (Fig. 9).

Quadripartition of the microspore mother cell is accomplished by centripetally advancing constriction furrows after the meiotic divisions are over. The mature pollen grains are two-celled and tricolpate without any characteristic sculpturings on the exine (Fig. 10).

Of the three layers of the young anther wall (Fig. 7), the innermost disintegrates during development, so that the wall of the mature anther consists of two layers — the epidermis and endothecium although not all cells of the latter develop the characteristic fibrous thickenings (Fig. 9). The epidermis becomes conspicuous because of dark brown inclusions. Whether the same is also true of *Wormia suffruticosa* (Paetow, 1931) remain to be investigated.

Persistence of the epidermis as the more dominant layer of the anther wall as in *Acrotrema* appears to be a comparatively rare feature among flowering plants. It would be interesting to know whether other genera of the Dilleniaceae exhibit this feature.

Endosperm and Embryo—During fertilization one of the synergids is destroyed by the entry and rupture of the pollen tube, while the other persists for some time (Fig. 11). The primary endosperm nucleus divides earlier than the zygote. The initial divisions, which take place in rather quick succession, are not accompanied by wall formation. The nuclei become arranged along the periphery of the widening embryo sac (Fig. 19). However, during slightly later stages, there is a relatively denser aggregation of nuclei and cytoplasm at the chalazal and micropylar ends, although the chalazal aggregation is rather poorly developed. In *Wormia* also Paetow noted a polar aggregation of the nuclear endosperm. His Fig. 17 shows that the chalazal aggregation is much more exaggerated than in *Acrotrema* (compare Paetow's Fig. 17 with our Fig. 20). Also, in *Wormia* the chalazal cytoplasm shows a number of vacuoles while the micropylar is completely devoid of them. In *Acrotrema*, however, vacuoles are absent both from the chalazal and

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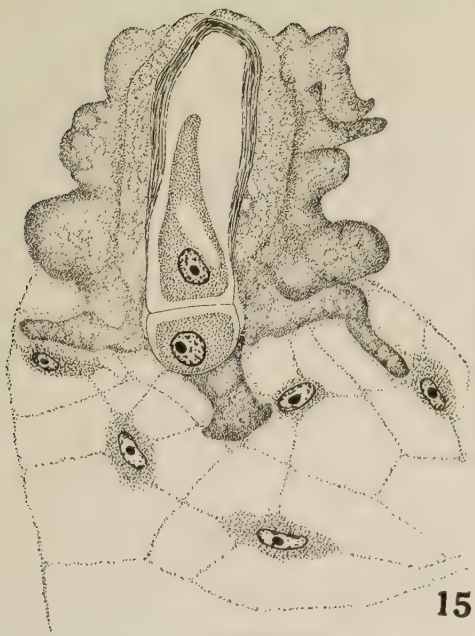
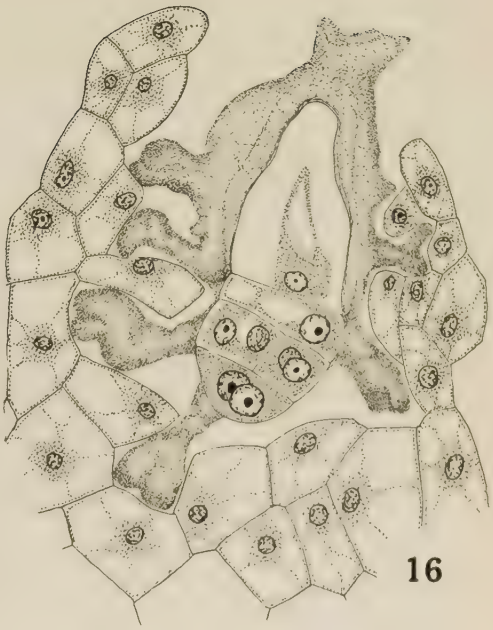
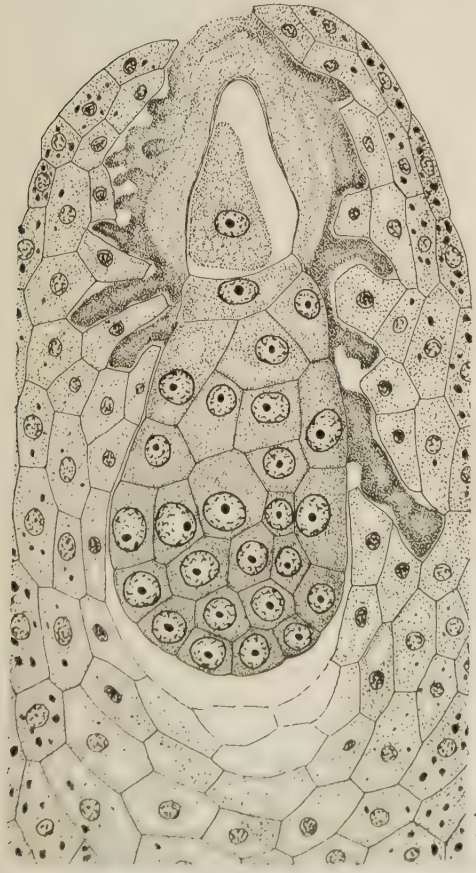
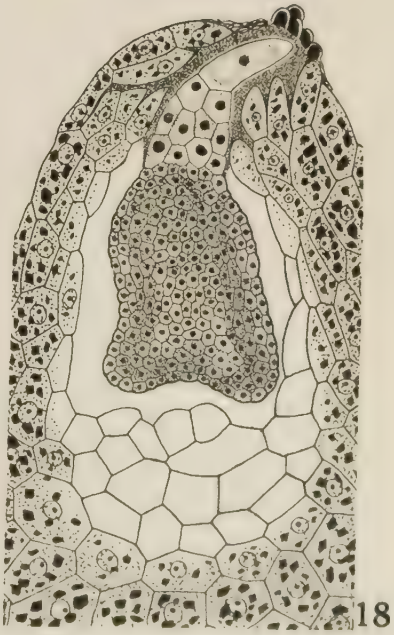
Figs. 15-18 — Fig. 15. Two-celled proembryo with its mantle. × 870. Fig. 16. Young proembryo pushing out of the mantle. × 870. Fig. 17. Later stage showing the beginning of degeneration of mantle. × 870. Fig. 18. Mature embryo, surrounding endosperm cells and remains of the mantle. × 390.





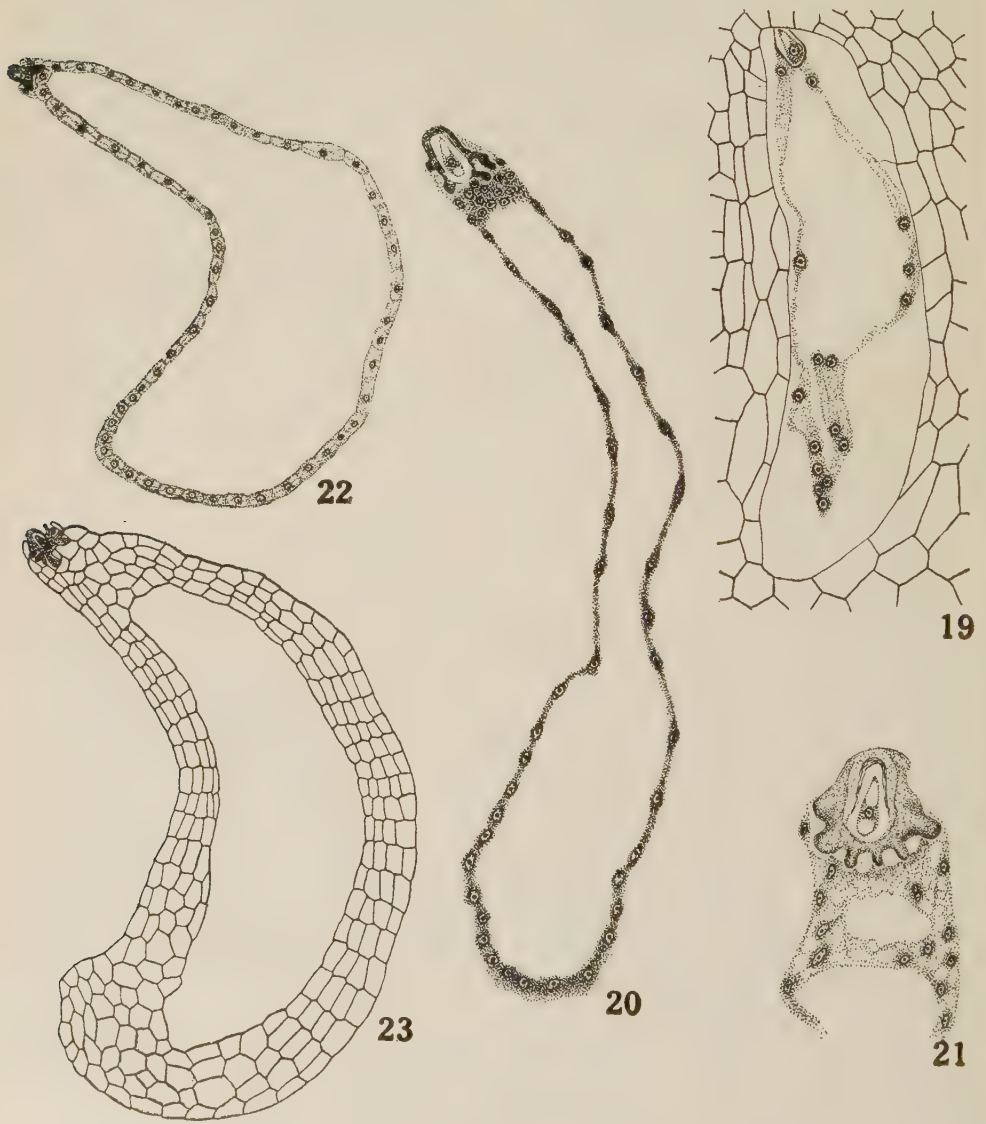




FIGS. 15-18.

micropylar aggregation only in the earlier stages (Fig. 20).

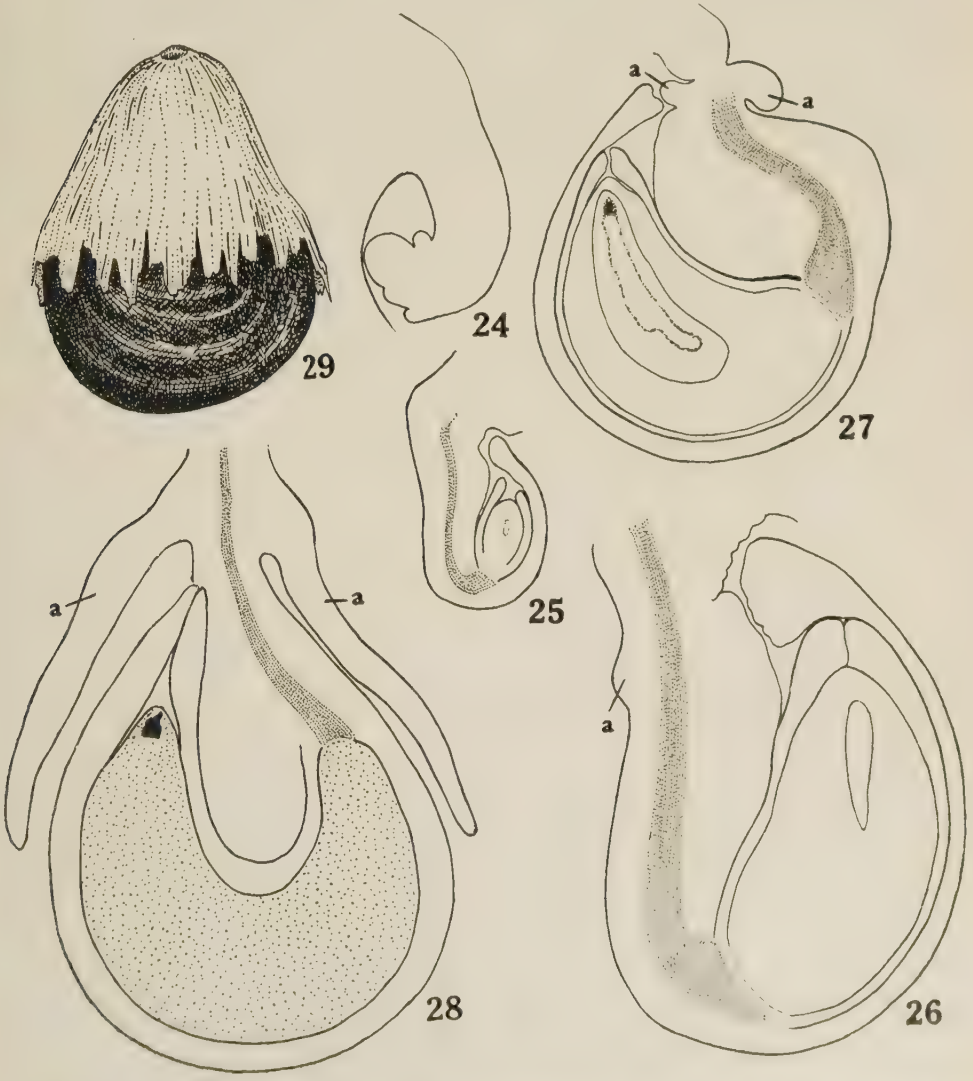
The endosperm protoplast that aggregates at the micropylar region forms a sheath around the zygote (Fig. 19). It must be emphasized that the zygotic wall is not a *true* wall and is not laid down as a result of phragmoplastic behaviour during gametogenesis. The physical nature of such a wall may be compared to a thickened plasma membrane which is plastic and is capable of undergoing reversible



FIGS. 19-23 — Fig. 19. An early stage of nuclear endosperm. × 350. Fig. 20. Later stage showing micropylar aggregation. × 175. Fig. 21. Vacuolation preceding wall formation in the micropylar aggregation of the endosperm. × 350. Fig. 22. An early stage in the formation of cell walls in the nuclear endosperm. × 95. Fig. 23. Later stage in the centripetal growth of the cellular endosperm. × 95.

structural changes during development. In view of this, we prefer to designate the limiting structure of the zygote as a *membrane*, rather than by the term wall. This membrane in *Acrotrema* is very tenuous in the early stages (Fig. 19).

At the time of fertilization, the amount of protoplasm in the embryo sac is very meagre, the maximum aggregation being confined to the neighbourhood of the secondary embryo sac nucleus. The same picture is presented soon after fertilization also (Fig. 11). Thus, it is obvious that zygotic protoplast is distinct from the endosperm protoplast. Even before the division of the primary endosperm nucleus, the endosperm cytoplasm spreads around the zygote so as to form a sheath, which is almost in contact with the zygotic membrane. By this time, the protoplast



Figs. 24-29 — (*a*, aril). Fig. 24. L.s. ovule primordium. × 195. Fig. 25. L.s. ovule at the megaspore mother cell stage. × 90. Fig. 26. At time of fertilization. × 90. Fig. 27. At time of nuclear endosperm. × 67. Fig. 28. L.s. mature seed. × 50. Fig. 29. Mature seed with aril × 40.

of the zygote undergoes a shrinkage, so that a space is created between the protoplast of the zygote and its membrane (Fig. 19).

The zygote begins to elongate towards the chalaza in the form of a narrow sac and its nucleus undergoes considerable enlargement (Fig. 12). Simultaneously, the zygotic membrane also shows very significant modifications. The outer surface of the membrane in the neighbourhood of the micropyle begins to swell in the form of weak undulations (Fig. 12). The swollen part of the zygotic membrane (in the initial as well as subsequent stages) appears more finely granular than the surrounding endosperm cytoplasm and shows a different staining reaction from the latter in that the zygotic membrane stains orange-red with erythrosin while the endosperm cytoplasm takes a brilliant crimson.

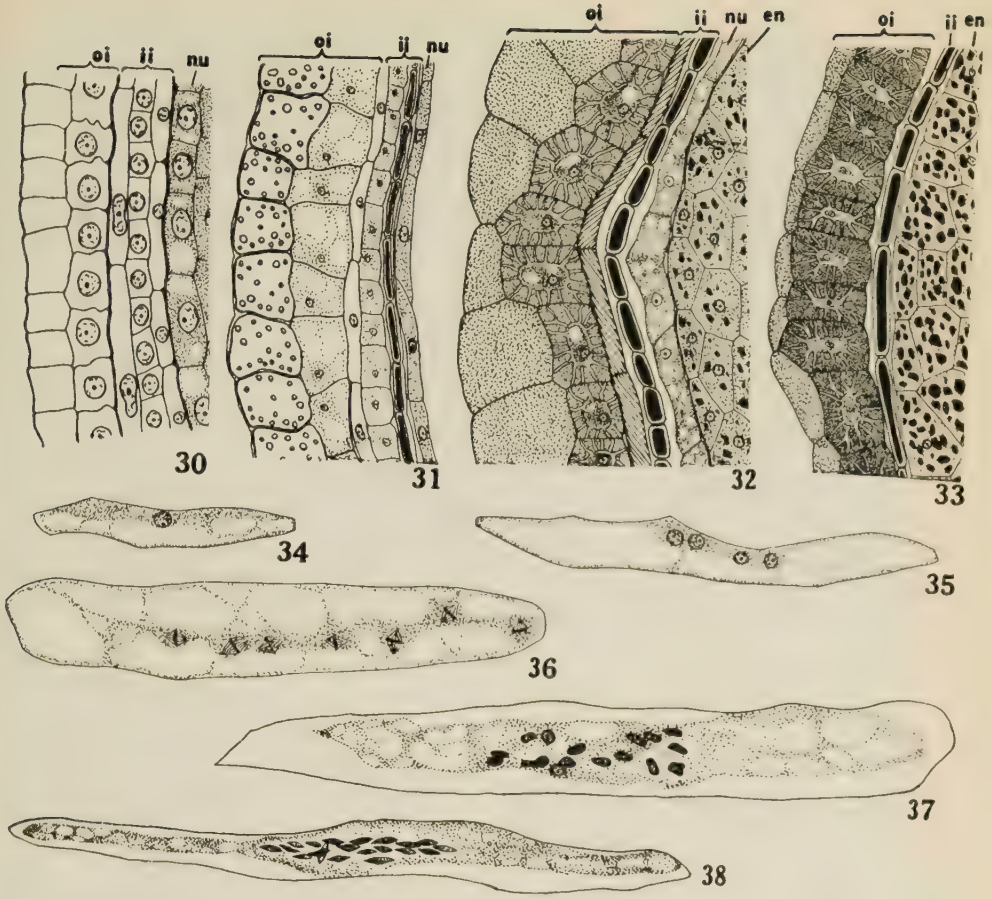
The swelling of the zygotic membrane progresses all along the sides (Figs. 13, 40). The swollen part, designated here as a mantle because of its modified morphological form, becomes distinguishable into two parts: the inner part, which is rendered gelatinous or mucilagenous and shows a lamellated structure, and the outer part which presents the appearance of a densely viscous cytoplasm (Figs. 13, 42). Gelatinization of the inner part of the zygotic mantle is first initiated at the micropylar end and gradually spreads along the sides (Figs. 13, 15).

The undulated swellings protrude in the form of finger-like stubs projecting into the surrounding cytoplasm in which free nuclear divisions have already taken place (Figs. 14, 41). It may be noted that the endosperm cytoplasm becomes thinned out in regions between the finger-like processes of the zygotic mantle (Figs. 14, 21, 41). Even after the maximum development of the zygotic mantle, the lower part of the original zygotic membrane remains unmodified (Fig. 14).

By the time the zygote nucleus has undergone the first division, the swellings of the mantle become still further differentiated in the form of numerous irregular finger-like projections. The latter are solid and more deeply staining towards the tips, and show a finely vacuolated pattern (Fig. 15). The first division of the zygote is transverse and results in the formation of a long, tubular basal cell, and a smaller apical cell (Fig. 15). The basal cell does not undergo further divisions. The terminal by successive divisions, gives rise to a mass of cells (the exact sequence of cell generations was not followed), which pushes itself against the tenuous unmodified part of the zygotic mantle (Figs. 16, 43). During later stages, due to the continued growth of the derivatives of the apical cell, the embryonal mass escapes out of the mantle and comes in immediate contact with the surrounding cellular endosperm (Figs. 17, 44, 45). The micropylar half of the embryo, however, remains enclosed by the mantle.

Fig. 20 represents a stage prior to wall formation in the endosperm. As already explained on a previous page, there is a conspicuous accumulation of endosperm nuclei and cytoplasm beneath the zygote. It may be mentioned in this connection that the cytoplasm in this region appears to be highly basichromatic, refractive, and coarsely granular. Perhaps it is due to these factors that we encountered great difficulty in obtaining proper differentiation between the nuclei and cytoplasm of this region. It is only with the Feulgen reaction that the presence of nuclei could be demonstrated with certainty. The endosperm aggregation in the neighbourhood of the zygote gradually begins to thin out before wall formation. The process of thinning out somewhat reflects the subsequent plane of deposition of the wall. In early stages, the thinning process is brought about between the nuclei by vacuoles. Gradually, groups of vacuoles fuse to form larger ones whereby the cytoplasm presents the appearance of a network of strands connecting the nuclei (Fig. 21). The strands that separate the adjacent nuclei form the basement of the so-called walls of the endosperm cells (Figs. 15, 16).

The endosperm at first becomes cellular in the micropylar part, and this differentiation gradually spreads towards the chalaza until all the peripheral endosperm nuclei become enclosed within cell walls (Fig. 22). Further increase in endosperm is accomplished through repeated



FIGS. 30-38 — (*en*, endosperm; *ii*, inner integument; *oi*, outer integument; *nu*, nucellus). Figs. 30-33. Stages in the differentiation of the seed-coat; for explanation see text. Fig. 30. × 710; others × 230. Figs. 34-38. Cells of the aril showing nuclear behaviour. × 230.

divisions of the peripheral endosperm layer, essentially by means of periclinal walls (Fig. 23). Finally, the entire cavity becomes filled with endosperm cells packed with fatty substances and other inclusions (Fig. 18). In the immediate neighbourhood of the embryo, however, the cells are depleted of their contents, a feature also seen in *Wormia* (Paetow, 1931).

After the endosperm in the micropylar part becomes cellular, the finger-like processes of the zygotic mantle begin to show increased activity. The finger-like processes become longer and their tips develop coralloid protuberances. Many of the processes are seen to penetrate the endosperm cells in an intercellular manner (Figs. 16, 17, 44, 45).

When the endosperm cells begin to show the accumulation of lipoidal and other inclusions, the processes of the zygotic mantle appear to become somewhat withered (Fig. 17). In mature seeds, the remains of the mantle may still be clearly seen in the neighbourhood of the suspensor region as amorphous streaks radiating between the endosperm cells (Fig. 18). The embryo is fully differentiated, but is quite small as compared with the endosperm (Fig. 28).

FIGS. 39-41 — Fig. 39. Photograph of *Acrotrema* plants in their natural habitat. $\times \frac{1}{6}$. Figs. 40, 41. Successive stages in the development of the zygotic mantle. $\times$ 965 and 715 respectively.

A mantle-like modification of the zygotic membrane has not been recorded anywhere except in the family Dilleniaceae. Paetow (1931), who first noted the presence of this feature in *Wormia*, surmises that this structure may have a haustorial or mechanical function. In our opinion, the former function appears to be more probable.

In regard to the morphology of the mantle, Paetow casually remarks that it takes its origin from the endosperm cytoplasm that lies in the immediate neighbourhood of the zygote. Our observations, on the other hand, indicate that the mantle is a modification of the zygotic membrane itself (the so-called wall of the zygote). The reasons for this are as follows:

1. The zygotic membrane is highly tenuous and plastic, and sometimes appears only as the hardened periphery of the zygotic protoplasm.

2. At the time of elongation of the zygote, the protoplast of the cell conspicuously shrinks away from the membrane.

3. The cytoplasm of the endosperm surrounding the zygote is of a coarser texture whereby the zygotic membrane stands out prominently from the surrounding cytoplasm.

4. The initial localized swellings of the zygotic membrane take place independently of the surrounding endosperm cytoplasm.

5. The gelatinization of the inner part of the zygotic mantle is characteristic of a membrane or wall, rather than of a formless cytoplasm.

Seed — The nucellar tissue undergoes rapid disorganization after fertilization. Even before the endosperm reaches the cellular stage, the entire nucellus except the outermost layer and a small portion at the chalaza get completely disorganized. The persisting nucellar cells towards the sides undergo much elongation (Figs. 31, 32). However, when the seed becomes fully mature, this layer is crushed and destroyed (Fig. 33). These features are also shared by *Wormia* (Paetow, 1931).

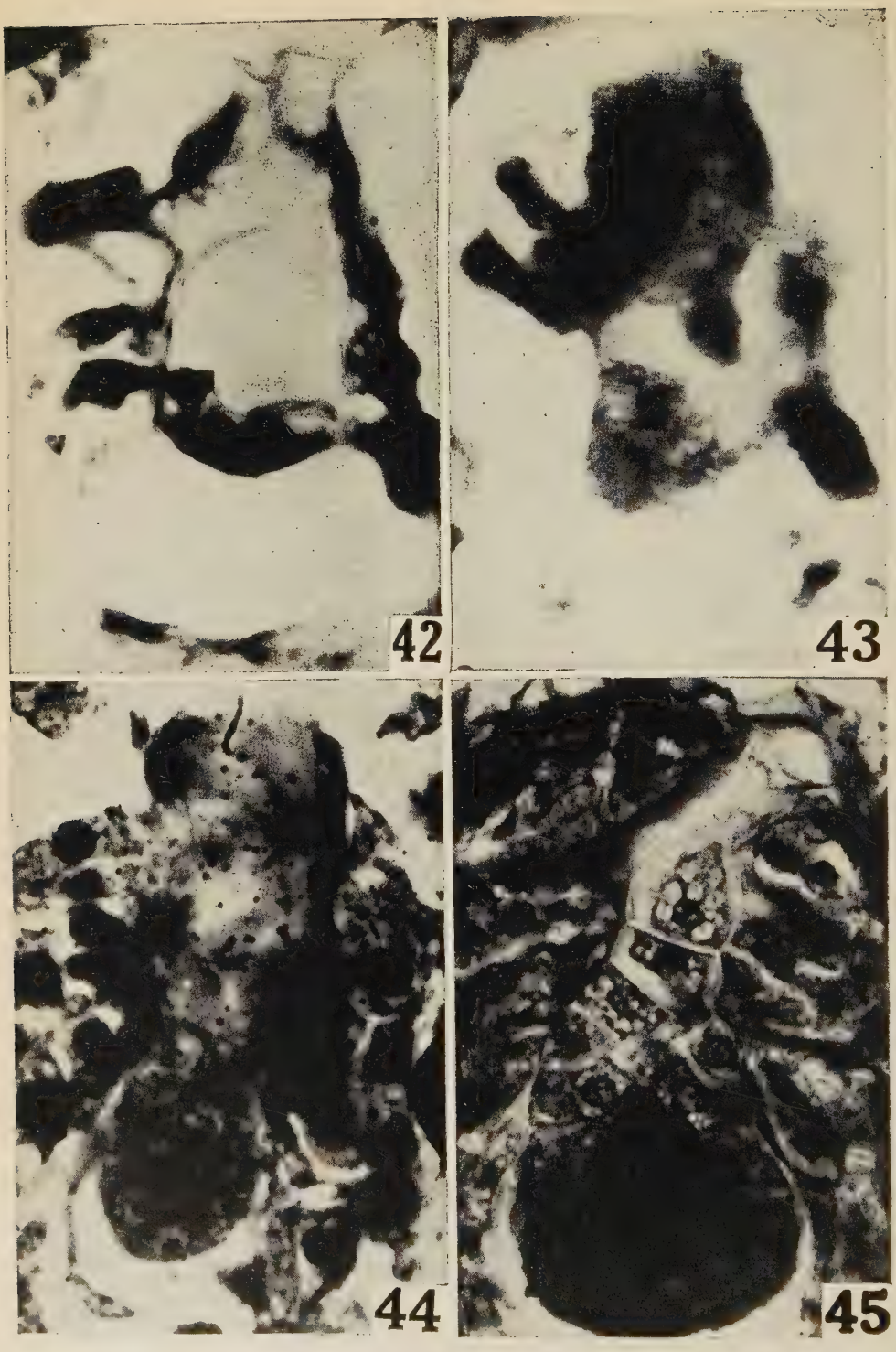
At the time of fertilization, the outer integument consists of two cell layers and the inner of three (Fig. 30). During the development of the seed, the outer layer of the outer integument accumulates globules of tanniferous material (Fig. 31), which gradually increase in quantity so as to fill the cells completely (Figs. 32, 33). Groups of such cells become enlarged and this feature gives a sort of mildly mottled appearance to the surface of the seed. In later stages, the cell layer as a whole becomes compressed (Fig. 33). The inner layer of the outer integument undergoes excessive sclerification and hardening with numerous branching pits (Figs. 32, 33).

The cells of the innermost layer of the inner integument accumulate brownish contents soon after fertilization and persist in the same state even in the mature seed (Figs. 31-33). The cells of the outermost layer of the inner integument undergo much elongation and during later stages develop spiral thickenings (Figs. 31, 32). However, as the seed matures, this layer gets almost completely crushed and destroyed (Fig. 33). The middle layer of the inner integument also undergoes early degeneration. Thus, in the fully mature seed, only the innermost tannin-filled layer of the inner integument and both the layers of the outer integument persist in the modified form as explained above.

Due to the pronounced amphitropous curvature of the seed, the horse-shoe-shaped space between the micropylar and chalazal ends becomes filled with the raphe tissue (Figs. 25-28). The cells of this tissue are remarkable in showing a multinucleate condition due to one or two free nuclear divisions.

The seed coat of *Wormia*, as described by Paetow (1931), is quite similar to what is found in *Acrotrema*. But his Fig. 19, which does not probably represent the fully mature state of the seed coat, fails to show the innermost layer of tannin-filled cells, although he mentions the presence of this feature in the text.

Aril — The primordium of the aril (*a* in Fig. 26) arises as a ring-like protuberance around the base of the funicle even before fertilization. However, the primordium becomes active only during the post-fertilization development. The cells multiply, the aril grows along the surface of the seed and at maturity encloses the micropylar and chalazal ends (Figs. 28, 29). Its cells are large and elongate along the vertical axis.



FIGS. 42-45.

To begin with, the aril consists of uninucleate cells (Fig. 34). During development, the nucleus undergoes four successive and synchronous free nuclear divisions so that 16 nuclei are formed. Occasionally, a fifth division may take place, which may also be synchronous or may affect only a few of the nuclei. In the latter case, the total number of nuclei per cell would lie between 16 and 32 (Figs. 35-37). These nuclei gradually move towards the centre of the cell and show a pronounced tendency to lie close together (Figs. 37, 38). In the mature state of the seed, the nuclei become distorted into spindle-shaped amorphous bodies (Fig. 38). The cytoplasm of the cells become increasingly coarser and more uniformly distributed during later stages of ontogeny. The multinucleate condition of the cells of the aril as also of the raphe tissue appears to be a feature not recorded elsewhere.

Summary

The ovule is typically anatropous at the time of fertilization, but becomes completely amphitropous during post-fertilization development.

Both the primary parietal cell and the nucellar epidermis take part in the building up of the parietal tissue. The development of the female gametophyte conforms to the *Polygonum* type.

The anther tapetum is binucleate and secretory. Quadrpartition of the microspore mother cells is accomplished by furrowing. The pollen grains are two-celled at the time of shedding. The epidermis as well as the partially differentiated endothecium persist in the mature anther.

The endosperm is of nuclear type. The endosperm nuclei are distributed uniformly in a single layer along the periphery of the embryo sac, excepting in the neighbourhood of the zygote, where there is a denser accumulation. Walls begin to appear at this stage and the subsequent development of the tissue is largely through periclinal cell divisions.

The most remarkable feature during the post-fertilization development is the organization of a mantle by the zygotic membrane. The external surface of the membrane swells and grows out in the form of coralloid protuberances between the surrounding endosperm cells. The mantle and its protuberances appear essentially cytoplasmic as evidenced by its microscopic structure. The inner surface of the mantle becomes gelatinous and lamellated.

In the mature seed the size of the fully differentiated embryo is significantly small as compared with the amount of endosperm. Both the layers of the outer integument and the innermost layer of the inner integument undergo structural changes to constitute the seed coat.

The aril differentiates from the funicle and covers half the seed in the mature condition. A feature of unusual interest in the cells of the aril is their coenocytic nature. As many as 32 nuclei were counted in the cells.

We thank Professor A. Abraham, Department of Botany, University of Travancore, for his kind help in the collection of material, and Mr. S. Rajan, Presidency College, Madras, for assistance in photomicrography. We tender our grateful thanks to Professor P. Maheshwari, Department of Botany, University of Delhi, for the loan of relevant literature.

Postscript — After sending the paper to press, we saw A. Nagaraja Rao's paper on the gametophytes *Dillenia pentagyna* (*Curr. Sci.* **24**: 62, 1955). His account agrees in general with our observations on the corresponding structures in *Acrotrema arnottianum*.

Figs. 42-45 — Fig. 42. Obliquely cut view of the zygotic mantle showing the denser projections from the periphery and gelatinization of the inner surface. Fig. 43. Young proembryo pushing out of the mantle; embryo cut obliquely. Fig. 44. L.s. young embryo and its surrounding regions at the time of maximum activity of the mantle. The coralloid projections are clearly seen. Fig. 45. A later stage, when the mantle begins to exhibit an amorphous texture. Figs. 42-44. × 1,000; Fig 45. × 900.

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STUDIES OF THE GARRYACEAE — I. THE COMPARATIVE MORPHOLOGY AND PHYLOGENY

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Introduction

One of the most fascinating and seemingly inscrutable mysteries of phylogenetic botany is the evolutionary origin of the Angiospermae. Although many hypotheses have been offered, few of them are credible and none has received convincing proof. It has become clear that a natural classification of the plant kingdom, especially of the Angiospermae, must be founded on a synthesis of the evidence taken from all available sources of botanical knowledge (Bailey, 1944a, 1951; Lawrence, 1951; Tippo, 1946) rather than to rely, as in the past, upon the morphology of the flower alone or some other single type of evidence. During recent decades, the study of the secondary xylem of many woody dicotyledons has revealed considerable evidence for the relationships of certain angiospermous families, such as the Himantandraceae (Bailey, Nast & Smith, 1943), the Casuarinaceae (Moseley, 1948), and the Moraceae (Tippo, 1938). The role of wood anatomy in phylogeny and the relatively unbiased nature of the evidence revealed have been stressed by several systematists (Bailey, 1951; Record, 1934b; Tippo, 1946; Turrill, 1942).

Studies of the morphology and vascular systems of the inflorescences and flowers have also offered important evidence for the relative primitiveness or specialization of certain phylogenetically important families (e.g. Abbe, 1935; Bechtel, 1921; Manning, 1948). Many other fields of study are helpful and information from them, often readily available, should be consulted in any taxonomic or phylogenetic investigation.

The Garryaceae were chosen for study because of the extraordinary difficulty of relating the taxon to other families. *Garrya* Douglas ex Lindley in Botanical Register 20: t. 1686 (1834) is the only genus of the family. The species are distributed from Guatemala and Mexico through southern and central Texas, New Mexico, Arizona and Nevada, to California, Oregon, and Utah. One species, *G. fadyena*, is found on the island of Jamaica and the eastern portion of Cuba.

Lack of knowledge of the family is partly responsible for the confused ideas of its systematic position. There has been only one thorough morphological study of the family, and even this study is incomplete for lack of material (Hallock, 1930). The amentiferous inflorescences bestow phylogenetic significance upon this family, for many amentiferous taxa are considered primitive angiosperms or derivatives of ancient transitional forms

between coniferous or ephedroid ancestors and more recent and advanced angiosperms (Engler & Diels, 1936; Hagerup, 1934, 1936, 1938; Hjelmqvist, 1948). Other authors, as Bessey (1915) and Hutchinson (1926), consider the amentiferous families as specialized taxa derived from other groups of a more primitive nature.

The anatomies of the secondary xylem, secondary phloem, and the node have been investigated and described by the joint effort of the writers. Evidence of phylogenetic significance obtained from the original study has been considered in conjunction with other available evidence by the senior author to gain further insight into the natural relationships of the Garryaceae and to cast some light upon the problem of angiospermous origin.

Material and Methods

The Secondary Xylem — The secondary wood of 105 samples of 11 species was investigated. The clarification of the nomenclature of the species was sought principally in the "Index Kewensis" (Jackson *et al.*, 1893-1940) and in the works of Bacigalupi (1924), Eastwood (1903), McMinn (1951), and Standley (1920-1926). The limitations of Californian species is problematical. There follows a list of the species studied, their places of collection, and the number of samples investigated:

Garrya veatchii Kellog. (California) — 16.
G. flavescens, S. Wats. var. *pallida* Eastw. (California) — 24
G. elliptica, Dougl. (California) — 11
G. wrightii, Torr. (Arizona) — 12
G. buxifolia, A. Gray (California) — 11
G. fremontii, Torr. (California) — 10
G. congdonii, Eastw. (California) — 12
G. laurifolia, A. Gray (Mexico) — 3
G. lindheimeri, Torr. (Texas) — 2
G. fadyena, Hook (Jamaica) — 3
G. ovata, Benth. (Mexico) — 1

Herbarium specimens collected with each wood sample of the Californian and Arizonian species have been deposited in the herbarium of the University of California, Santa Barbara College. Herbarium specimens for samples of the other species may be found in the herbaria of Harvard and Yale universities and the Chicago Museum of Natural History which loaned the wood samples.

Six microscopic slides were prepared from each sample, according to standard methods (Wetmore, 1932). Sections were cut on a sliding microtome at 15 μ following softening in 48 per cent hydrofluoric acid. Sections were stained in Heidenhain's haematoxylin and safranin. A complete collection of slides is filed at the Department of Biological Sciences, University of California, Santa Barbara College, and representative collections have been accepted by the Harvard University Herbarium and the School of Forestry, Yale University. Macerations with Jeffrey's maceration fluid (Chamberlain, 1932) were made of all samples for length measurements of the imperforate and vessel elements. Sections and macerations were made from wood outside of the fifth increment, and, so far as known, samples were taken from relatively normal, straight stem wood well above soil level (Chalk, 1930; Desch, 1932b).

The terminology used in the anatomical descriptions complies with that approved by the Committee on Nomenclature, International Association of Wood Anatomists (1933). The choice of diagnostic characters to describe the secondary xylem was taken largely from the lists prepared by Record & Chattaway (1939) and Tippo (1941).

Due to the controversy over the definitions of imperforate elements, determinations of these elements were based upon both the standards set up for the delimitations of element types by Bailey (1936) and by Reinders (1935, 1951), and Varoissieau (1951). As no libriform wood fibres or other specialized types defined by either system of classification were found, little difficulty was encountered. The relative thickness of the walls of the imperforate elements has been classified (Chattaway, 1932) with reference to the radial wall, as follows:

Very thin — lumen much greater than the thickness of walls.

Thin — lumen greater than thickness of walls.

Thick — lumen much less than thickness of walls.

Very thick — lumen almost completely closed.

Twenty-five measurements per sample, or 2,625 measurements, of the diameters of the pit-pairs of imperforate elements, and the same number of the diameters of the pit-pairs of vessel elements, were made to give relative pit-pair size and to ascertain the classification of the imperforate elements. Length measurements of 5,250 imperforate elements were made on macerated material. Fifty measurements of imperforate cells were taken at random from each of the 105 samples of the 11 species. The length categories of the imperforate elements and vessel elements are (Committee on the Standardization of Terms of Cell Size, International Association of Wood Anatomists, 1937):

	Imperforate elements (μ)	*Vessel elements* (μ)
Extremely short	Less than 500	Less than 175
Very short	500-700	175-250
Moderately short	700-900	250-350
Medium-sized	900-1600	350-800
Moderately long	1600-2200	800-1100
Very long	2200-3000	1100-1900
Extremely long	Over 3000	Over 1900

A statistical study of the measurements was completed as suggested by Desch (1932a) and by Rendle & Clarke (1934a, 1934b).

The number of vessels were counted in 10 " high dry " microscopic fields, chosen at random, per sample (transverse sections), or a total of 1,050 counts, to determine the frequency of vessels per square millimeter. The classes utilized for describing the frequency of vessels are (Chattaway, 1932; Metcalfe & Chalk, 1950):

Few	0-10
Moderately numerous	10-20
Numerous	20-40
Very numerous	Over 40

Vessel element diameters were measured from transverse sections. Twenty-four elements per sample, chosen at random, were measured from slides of all 105 samples, or 2,520 measurements. The size classification for vessel diameters is (Chalk, 1938):

Extremely small	up to 25 μ
Very small	25-50
Moderately small	50-100
Medium sized	100-200
Moderately large	200-300
Very large	300-400
Extremely large	Over 400

A statistical analysis of the vessel diameter measurements is presented (Desch, 1932a; Rendle & Clarke, 1934a, 1934b).

The classification for scalariform perforation plates was proposed by Frost (1930b): few, 5 or fewer bars; intermediate, 5 to 15 bars; many, 15 or more bars. The openings (measured from bar to bar) of the scalariform perforation plates are classified as narrow, if they are 6 μ or less; or wide, if they are over 6 μ (Frost, 1930b). The arrangement of the intervascular pitting and of the pitting between vascular rays and vessels is described according to the familiar types: scalariform, transitional, opposite, and alternate (Committee on Nomenclature, International Association of Wood Anatomists, 1933; Frost, 1930b). The size classes for intervascular pit-pairs are (Record & Chattaway, 1939): minute (up to 4 μ diameter); small (4 to 7 μ); medium-sized (7 to 10 μ); large (10 to 15 μ); very large (over 15 μ).

The lengths of a total of 3,150 vessel elements were measured. Thirty measurements of elements from each of the 105 samples of the 11 species were taken at random (Chalk, 1930; Desch, 1932a). The measurement of each vessel element was taken from the tip of one " tail " to the tip of the other (Chalk & Chattaway, 1934, 1935; Committee on the Standardization of Terms of Cell Size, International Association of Wood Anatomists, 1937). A statistical study of the length measurements of the vessel elements is offered (Desch, 1932a; Rendle & Clarke, 1934a, 1934b).

The vascular rays have been classified according to the types described by Kribs (1935). The widths of the rays

have been measured in micra and by cells. Rays are described as fine with maximum width less than 50 μ; moderately wide, 50 to 100 μ; and broad, over 100 μ (Metcalfe & Chalk, 1950). The heights of the rays were measured in micra and the maximum heights noted (Metcalfe & Chalk, 1950).

The classification of wood parenchyma distribution types utilized here is a synthesis of the ones presented by Metcalfe & Chalk (1950) and Hess (1950) with the suggestions discussed by Normand (1951) and others at the international meeting in 1950 of the International Association of Wood Anatomists:

Apotracheal
- Diffuse
- Scattered diffuse
- Diffuse-in-aggregates
- Banded
- Marginal
- Terminal
- Initial
- Reticulate (artificial)

Paratracheal
- Scanty Paratracheal
- Vasicentric
- Aliform
- Confluent
- Unilaterally paratracheal
- Abaxial
- Adaxial

Plant anatomists over a period of four decades have determined the lines of phylogenetic specialization of the stele, particularly of the secondary xylem, mostly by methods based upon the work of Bailey & Tupper (1918). These authors made determinations of cell length of xylic elements in hundreds of species drawn from the major taxa of the vascular plants. Bailey & Tupper found that the tracheary elements are very long in the lower vascular plants, intermediate in length in the gymnosperms, and relatively short in the angiosperms. They also concluded that the specialization of the vessel element has been correlated with the reduction in length of the tracheary elements. Tippo (1946) and Turrill (1942) explain that directions of the lines of specialization have been established either by basing them upon paleobotanical, comparative morphological, and developmental studies of the principal vascular plant taxa, or, using the work of Bailey & Tupper (1918) as a foundation, by the methods of association and correlation described by Frost (1930a). Many anatomists (e.g. Bailey, 1944a, 1951, 1953; Chalk, 1937; Record, 1934b; Tippo, 1946) consider that the phylogenetic trends thus determined are sound, and formulated independently of preconceived theories of angiospermous classification or origin. The anatomist's contribution to the solution of a phylogenetic problem in the Angiospermae may be considered based upon evidence obtained by independent methods and hence relatively free of bias. Evidence obtained from study of the secondary xylem of a taxon, when coupled with the available evidence from other botanical research, may well determine the phylogenetic level and the systematic position of a taxon. The phylogenetic sequences appear in certain papers (Bailey, 1953; Moseley, 1948; Tippo, 1938, 1946).

The Secondary Phloem — Macerations were made of the bark of 3 samples of *G. flavescens* var. *pallida*, 3 of *G. veatchii*, 3 of *G. elliptica*, 2 of *G. wrightii*, 2 of *G. fremontii*, 2 of *G. buxifolia*, and 1 of *G. lindheimeri*. Microscopic slides with sections in the three planes of the phloem of 2 samples of *G. veatchii*, 1 of *G. flavescens* var. *pallida*, 2 of *G. elliptica*, 2 of *G. wrightii*, 2 of *G. fremontii*, 1 of *G. laurifolia*, and 1 of *G. lindheimeri* were studied.

The evolution of the secondary phloem of the Dicotyledoneae has been little studied and few evolutionary sequences are known with certainty. There is some evidence that certain evolutionary changes have occurred (Hemenway, 1913; Huber, 1939; MacDaniels, 1918). In the Dicotyledoneae, very long sieve elements not connected vertically to form tubes are primitive and sieve elements arranged vertically to form tubes are advanced. The former condition is rare in dicotyledons (Bailey & Swamy, 1949). Apparently, slender and moderately long sieve-tube elements which have oblique end walls with compound sieve plates and poorly specialized sieve areas on the side walls are primitive. Sieve-tube members

which are relatively broad and short, which have nearly transverse to transverse end-walls each with a simple, highly specialized sieve plate, and which have vestigial, non-functional or no sieve areas on the side-walls are specialized. Abundant evidence supporting such a phylogenetic sequence has been described for the Monocotyledoneae (Cheadle, 1948; Cheadle & Whitford, 1941). At present, there is little reason to think that phloic evolution in the dicotyledons has not been similar (Esau, 1953; Esau, Cheadle & Gifford, 1953).

Nodal Anatomy — It is thought that the double-trace, unilacunar condition is primitive (Marsden & Bailey, 1955) and that the trilacunar, unilacunar, and multilacunar types described by Sinnott (1914) are derived. In general, the trilacunar type is moderately advanced and the unilacunar and multilacunar conditions most specialized and derived from the trilacunar. The unilacunar in some cases, however, has been derived from the double-trace, unilacunar type.

Free-hand sections of the vegetative nodal regions of 7 species were prepared to determine the number of leaf-traces and leaf gaps. Material of the following species was examined: *G. veatchii*, *G. flavescens* var. *pallida*, *G. elliptica*, *G. wrightii*, *G. buxifolia*, *G. fremontii*, and *G. lindheimeri*.

The Anatomy of the Secondary Xylem

Growth rings are always present (Figs. 2, 5). Although they are usually clearly distinct, they are occasionally poorly defined or weak. Typically, the annual increment is relatively narrow, as growth is slow. This is correlated with the rather unfavourable growth conditions of the chaparral or similar situations in which the species grow.

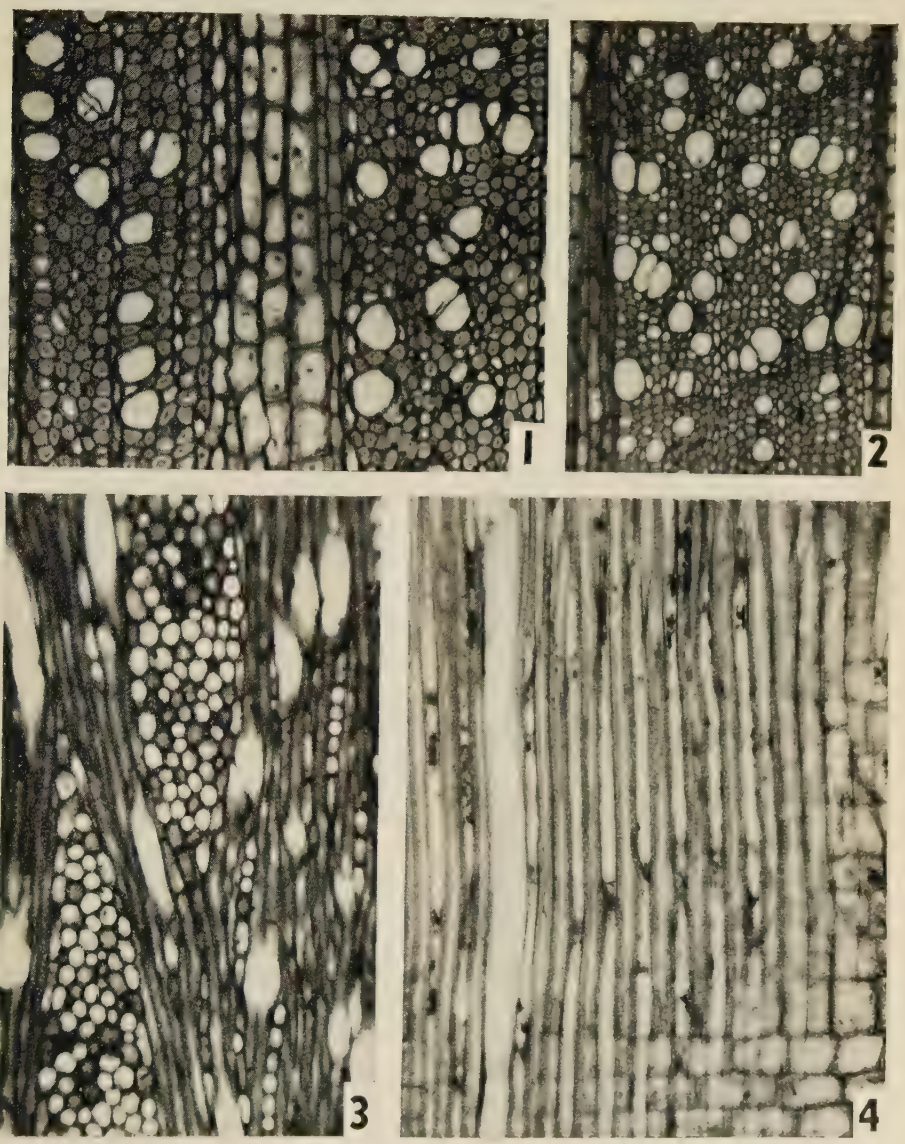
The Imperforate Elements — The imperforate elements are tracheids and fibre-tracheids. No libriform wood fibres were observed. In the early wood, tracheids predominate and tend to be more commonly aggregated around the vessel elements (Fig. 2) as vasicentric tracheids. Fibre-tracheids predominate in the late wood, but also occur in early wood in regions farthest from the vessels. No gelatinous or septate elements were observed. The walls of the imperforate elements vary from thin to very thick (classification previously listed) but are more frequently within the range from thin to thick (Figs. 1, 2, 5).

The horizontal diameters of the pit-pairs as seen in radial and tangential face views vary from 2 to 9 μ; the most frequent range is from 3·5 to 5 μ. The pit-pairs are bordered and generally circular but are occasionally oval. The inner apertures of all pit-pairs are elongated and those of a pit-pair are crossed. The elongated inner apertures do not ordinarily extend beyond the outline of the border.

Fifty length measurements at random of imperforate cells of each of the 105 samples (5,250 measurements) were taken. The length of these elements for the genus varies from 210 (extremely short) to 1,470 μ (medium sized). The most frequent range is from 550 (very short) to 900 μ (moderately short) and the mean for the genus is $762 \pm 2{\cdot}9$ μ (moderately short) with a standard deviation of $215 \pm 2{\cdot}1$. The proportions of elements with spiral thickenings is quite variable from sample to sample. In only a few samples of certain species (*G. veatchii*, *G. flavescens* var. *pallida*, *G. elliptica*, *G. fremontii*, *G. congdonii*) are spiral thickenings to be found in all the imperforate elements. In some samples of a species, most elements have spiral thickenings. There is no correlation with early or late wood, nor is there any correlation with the type of element. Occasionally, the thickening is obviously a double one.

Vessels — The number of vessels per square millimeter in transverse aspect was calculated from 10 microscopic fields of all samples (1,050 measurements). The number varies from 24 (numerous) to 134 (very numerous); the most frequent range is 48 to 83 (very numerous) and the mean is 64. Vessel distribution is predominantly solitary (Figs. 1, 2, 5), but cluster (Figs. 1, 2) and chain (Figs. 1, 5) arrangements also occur sparingly. Quite rarely, multiples of 2 or 3 vessels occur (Fig. 5), but rarely does more

than 1 or 2 multiples occur per microscopic field. Pore multiples were seen in 26 samples of the 105, and in all the species studied except *G. veatchii*, *G. flavescens*



FIGS. 1-4 — Secondary xylem of *Garrya*. Fig. 1. T.s. *G. buxifolia* to show thin- to thick-walled imperforate elements; solitary vessels, pore chains, and pore clusters; angular vessels; diffuse, diffuse-in-aggregate, and scanty paratracheal wood parenchyma. × 200. Fig. 2. T.s. *G. buxifolia* to demonstrate growth rings; thin- to thick-walled imperforate elements; vasicentric tracheids; solitary vessels, and pore clusters; angular vessel outline; ring porosity; diffuse, banded, and reticulate wood parenchyma. × 145. Fig. 3. Tangential section of *G. elliptica* showing heterogeneous Type II B vascular rays, sheath cells, thickened walls of ray cells. × 145. Fig. 4. Radial section of *G. elliptica* demonstrating tile cells, thickened walls of ray cells, fusiform and strand wood parenchyma, scanty paratracheal wood parenchyma. × 245.

var. *pallida*, *G. lindheimeri*, and *G. fadyena*. The wood is clearly ring porous (Figs. 2, 5) in most samples, but occasionally ring porosity is indefinite (semi-ring porous). In *G. buxifolia* and *G. laurifolia*, especially, the wood has ill defined ring porosity and is diffuse porous in some samples. The ring porosity is distinguishable largely by a reduction in the size of the vessels in the late wood rather than by a reduction in the number of vessels (Figs. 2, 5).

In all species, the vessels are strikingly angular in cross-sectional aspect (Figs. 1, 2, 5). Exceptions were observed in a few samples of *G. veatchii* and *G. flavescens* var. *pallida* where the vessels were circular or nearly so. The transverse-sectional diameters of 24 vessel elements from each of all samples, or 2,520 measurements, were taken. The diameters range from 14 μ (extremely small) to 64 μ (moderately small), and vary most frequently within the very small category (25 to 45 μ). The mean diameter for *Garrya*, on the basis of these measurements, is 34·2 ± 0·24 μ (standard deviation 11·6 ± 0·17, very small).

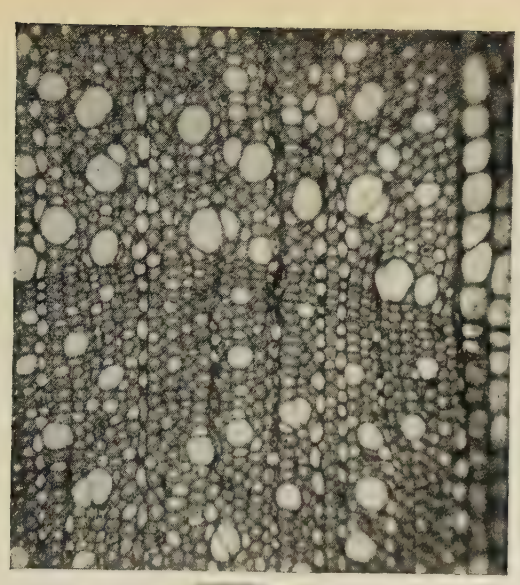
Perforation plates in the end walls of the vessel elements are of the scalariform type. No other types were observed. The number of bars in the perforation plates varies from 1 to 13 (few to intermediate), although rarely exceeding 10. The number of bars varies most frequently from 4 (few) to 7 (intermediate). The widths of the openings in the perforation plates vary from 5 (narrow) to 20 μ (wide) but measure most frequently between 10 to 12 μ (wide). No borders occur around the perforations. The end walls of the vessel elements in tangential aspect are generally quite oblique (Fig. 6) from 50 to 70° off the vertical, but vary a great deal from nearly transverse (horizontal) (20°) to very oblique (85°).

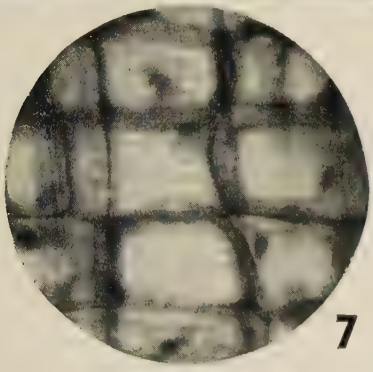
Intervascular pitting of the vessels varies from opposite to alternate (Fig. 9). In occasional samples, the alternate arrangement predominates. In two samples of *G. wrightii*, 1 of *G. buxifolia*, and 4 of *G. fremontii*, the transitional arrangement was rarely observed. The size of the intervascular pit-pairs ranges from 2 (minute) to 12 μ (very large), but ranges most frequently from 4 to 6 μ (medium-sized). Ten pit-pairs from each of the 105 samples were measured. The intervascular pit-pairs are predominantly circular (Fig. 9) in outline but occasionally oval to elongate pitting was observed. Generally, the pit-pairs have elongated and crossed apertures which in face view do not extend beyond the outline of the pit-border. The pitting between vessel elements and vascular ray cells is variable. Opposite and alternate pitting (Fig. 8) is most common, but transitional (Fig. 7) pitting was found quite frequently in most samples of all species except *G. fadyena* and *G. lindheimeri*. The ray-vessel pitting is bordered, not simple (Fig. 7).

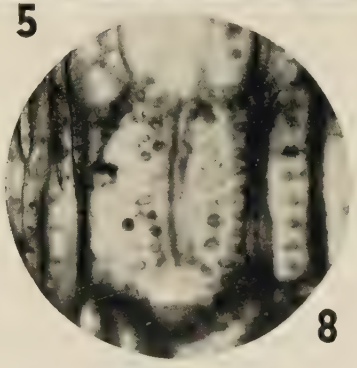
Thirty length measurements at random of vessel elements of each of the 105 samples (3,150 measurements) were taken. The lengths of the elements from the tip of one " tail " to the other varies from 151 (extremely short) to 1,168 μ (very (long). The most frequent range is from 412 to 630 μ (medium-sized) and the mean for the genus is 562 × 1·9 μ (standard deviation 104·5 ± 1·3) (medium-sized class). Spiral thickenings occur in all vessel elements.

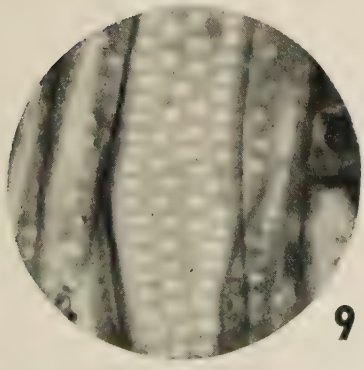
Vascular Rays — The frequency of rays across a tangential section is from 3 to 9 per millimeter and more commonly from 5 to 7 per millimeter. The vascular rays are both uniseriate and multiseriate (Figs. 3, 6). No compound rays occur, but definite indications of the aggregation of rays were observed with the production of aggregate rays in some samples of all species studied except *G. elliptica*. They are rare in *G. veatchii*.

The ray type is not constant, but varies from Heterogeneous Types IIA (Fig. 6) to IIB (Fig. 3) (Kribs, 1935). This variation occurs even among samples of the same species, although certain species have predominantly one or the other. For the samples studied, the rays of *G. veatchii* and *G. congdonii* are usually Heterogeneous Type IIA, whereas the rays of *G. elliptica*, and *G. buxifolia* are entirely Heterogeneous Type IIB (Fig. 3). The 24 samples of *G. flavescens* var. *pallida* and 12 samples of *G. wrightii* revealed transitional states with both Heterogeneous Types IIA and IIB in evidence.









FIGS. 5-10 — Secondary xylem of *Garrya*. Fig. 5. T.s. *G. flavescens* var. *pallida* to show growth rings; thin- to thick-walled imperforate elements; solitary angular vessels, pore multiples, and pore chains; ring porosity; tile cells; diffuse, diffuse-in-aggregate, and scanty paratracheal wood parenchyma. × 200. Fig. 6. Tangential section of *G. laurifolia* to show oblique vessel element end walls, Heterogeneous Type II A vascular rays, sheath cells, uniseriate rays, typical multiseriate rays 3-8 cells wide, strand parenchyma. × 145. Fig. 7. Radial section of *G. flavescens* var. *pallida* showing transitional to opposite ray-vessel pitting. × 360. Fig. 8. Radial section of *G. fremontii* demonstrating alternate ray-vessel pitting. × 360. Fig. 9. Tangential section of *G. fremontii* showing opposite to alternate intervascular pitting. × 360. Fig. 10. Radial section of *G. elliptica* showing fusiform parenchyma cells, same as Fig. 4. × 360

The multiseriate vascular rays vary within the genus from 8 to 310 μ wide (fine to broad) or from 2 to 18 cells wide (measured horizontally in tangential sections). The majority of the multiseriate rays vary within the range of 60 to 145 μ (moderately wide to broad) or from 3 to 8 cells (Figs. 3, 6). The uniseriate rays are from 4 to 8 μ in width (fine). The uniseriate rays vary in height from 20 to 720 μ, usually from 125 to 330 μ. In number of cells, they vary from 1 to 18 cells, typically from 3 to 8 (Fig. 3). The multiseriate rays are quite tall, 100 to 575 μ, but commonly from 420 to 165 μ.

Pitting between vascular ray cells and wood parenchyma cells is usually clustered in all species. The ray cells have secondary cell walls (Figs. 3, 4) which are lignified. No crystals were seen in the wood ray cells nor had any cells become sclerotic. Sheath cells (Figs. 3, 6) were very common in all species, although they did not occur on all rays throughout a tangential section of a sample. Tile cells are commonly present, both at the margins and within the body of the ray. They seem to be poorly differentiated, however, as though not fully evolved. They are lacking, apparently, in *G. elliptica*.

Wood Parenchyma — Both strand (Figs. 4, 6) and fusiform (Figs. 4, 10) types of wood parenchyma occur in most of the species examined. In addition, transitional conditions between the strand and fusiform types occur in those species with fusiform parenchyma. Strand parenchyma occurs in all of the species rather abundantly, but is occasionally absent in certain samples. Fusiform parenchyma occurs in all species except *G. buxifolia*. The fusiform type is frequently accompanied (in about one-half the samples) by parenchyma transitional between fusiform and strand types. Apparently, the cambial derivative, giving rise to such a transitional type, must have divided only once or twice to produce the intermediate condition.

Most of the wood parenchyma is apotracheal and occurs throughout the species studied as diffuse (Figs. 1, 2, 5) and diffuse-in-aggregate (Figs. 1, 5). Banded parenchyma (Fig. 2) also occurs in variable quantities: rare to fairly common in *G. fremontii*; common in some samples, but absent in others of *G. flavescens* var. *pallida*; common in all others. The banded parenchyma is abundant enough in *G. elliptica*, *G. wrightii*, *G. buxifolia* (Fig. 2), *G. congdonii*, and *G. laurifolia* to form a weak reticulate arrangement with the rays. These species should be described as having reticulate wood parenchyma, although this category is not a natural one. A small amount of paratracheal parenchyma (Figs. 1, 4) is found in all species, more than might normally occur in a strictly apotracheal wood. This parenchyma may be described as scanty paratracheal.

The Anatomy of the Secondary Phloem

The secondary phloem is fairly typical of woody dicotyledons and is composed of sieve-tubes, companion cells, phloem parenchyma, phloem rays, and phloem fibres. The vascular rays are of both uniseriate and multiseriate types. The multiseriate rays enlarge tangentially by radial divisions of the cells with subsequent tangential enlargement as the rays are pushed out by cambial activity. The large rays thus divide up the remainder of the phloem into wedge-shaped masses as in *Tilia* (Jeffrey, 1917) and many other woody dicotyledons. In the uniseriate rays and in the proximal portions of the larger rays, the ray cells are of two types: central cubical cells and marginal cells with their long axes vertically oriented. Occasional and scattered ray cells become sclerotic; or entire rays or large portions of them, especially the multiseriates, may become sclerotic. Large druses are formed very rarely in the ray cells.

The phloem fibres are very thick-walled with the lumen small or absent. They vary from 300 to 990 μ in length. They occur usually in tangential bands, 3-4 cells wide radially, alternating with the more delicate cells. The bands may extend from ray to ray, thus enclosing the conducting and parenchymatous cells. However, in some species there is considerable variation from small clusters to large

bands. In *G. laurifolia*, the fibres are largely solitary and scattered or in bands 1 cell wide radially. In *G. lindheimeri*, the fibres are solitary and rare to absent.

The sieve tubes and phloem parenchyma are arranged very regularly in radial rows. They are rectangular in cross-section, with the longer axis tangentially oriented. The sieve tube elements vary from 120 to 400 μ in length and have steeply oblique walls at 5°-80° off the vertical. Apparently, sieve areas are functional on both side-walls and end walls, but those on the end walls are more specialized, larger, and grouped into scalariform compound sieve-plates. The compound sieve-plates have from 3 to 6 sieve areas. This type of sieve element is considered moderately specialized. The companion cells were difficult to study and little is known of them. The phloem parenchyma cells are arranged in clusters or tangential bands. They are typically of the strand type but some are fusiform, similar to the strand and fusiform types, respectively, of the secondary xylem.

Nodal Anatomy

Leaves are sub-opposite and each leaf receives three traces, a central trace, and two laterals. There is a leaf-gap for each trace. The central trace to a leaf bends outwardly to the leaf base first; next, the lateral trace, counter-clockwise to the main trace, leaves the stele; and finally, the lateral trace, clockwise from the central trace, moves out. Externally, the leaves appear to be opposite. The vascular supply to one leaf, however, becomes distinct from the stele slightly lower than the supply to the opposite leaf. This relationship remains the same until the leaf petioles are distinct. This indicates that probably the present sub-opposite phyllotaxy was derived from a spiral arrangement. This information substantiates and amplifies that provided by Sinnott (1914).

Discussion

The Present Systematic Position of the Garryaceae — The relationship of the Garryaceae has been problematical and no systematist has appeared certain of the family's taxonomic position. Relationship has been indicated with five widely separated taxa: the Amentiferae, Celastrales, Umbelliflorae, Rubiales, and Hamamelidaceae. [The delimitation of each order and family herein discussed is arbitrarily that of Engler & Diels, 1936, except the taxa Celastrales and Amentiferae. The Celastrales represents the subordinal Celastrineae of Engler & Diels, 1936; the Amentiferae is used in the sense of Hutchinson (1926).]

Many authors of systematic works have placed the family in the artificial taxon called the Amentaceae or Amentiferae. Engler & Diels (1936) placed the Garryaceae as the fifth order, Garryales, of the Dicotyledoneae, among the monochlamydeous orders. They considered the monochlamydeous groups relatively primitive members of the Angiospermae, transitional between the more primitive achlamydeous groups and the derived dichlamydeous, polypetalous taxa.

Engler (1897) apparently did not consider the Amentiferae as a natural group, but felt that each order had an independent origin from a hypothetical taxon, the Protangiospermae. Nevertheless, Engler believed that dichlamydeous, polypetalous angiosperms arose from achlamydeous, amentiferous groups, not unlike the living Amentiferae, and that monochlamydy was a transitional stage. Faure (1924) in an anatomical study of the Cornaceae placed the Garryaceae between the Piperaceae and the Chloranthaceae. Lawrence (1951) considered that the Garryaceae were more closely related with the Umbelliflorae, but arbitrarily followed Engler & Diels (1936). Lindley (1853) was uncertain and related *Garrya* to amentaceous taxa, as well as to the Chloranthaceae and the Euphorbiaceae. Rendle (1925) followed the principles of Engler (1897) closely, but moved the Garryales up to second place in the Monochlamydeae preceded by only Salicales. A similar monochlamydeous position was assigned the family by Wettstein (1935) and Pulle (1937) and tentatively suggested by Coulter & Evans (1890). The principal reasons for grouping the Garryaceae with Amentiferae are the pendulous catkin or

catkin-like, unisexual inflorescences; 2-carpellate, 2-styled gynoecium; hypogyny, and monochlamydeous male flowers and achlamydeous female flowers.

Garrya has also been placed in proximity to the Umbelliflorae. Certain authors have included *Garrya* within Cornaceae along with *Cornus*, *Aucuba*, etc. (Baillon, 1867-1895; Bessey, 1915; Hallier, 1912; Warming, 1904; Wangerin, 1910). Other authors have held that the genus is closely related to the Cornaceae, but have removed the genus from the Cornaceae to its own family within the Umbelliflorae (Umbellales), next to Cornaceae (Hallock, 1930; Hjelmqvist, 1948). Adams (1949), who studied the anatomy of the Cornaceae, excluded *Garrya* from the family; but he apparently felt on the basis of work then in progress (1951, personal communication) that the Garryaceae should remain within the umbellalian complex.

It is thought that the authors who have related *Garrya* to Cornaceae may have done so on the assumption that *Garrya* is epigynous and not hypogynous, and has been derived from dichlamydeous ancestors. The pistillate flowers reputedly achlamydeous and hypogynous (Engler & Diels, 1936; Rendle, 1925; Lawrence, 1951) has at least a single perianth whorl represented by tiny lobes on top of the ovary in a young stage of development (Baillon, 1877, 1887; Faure, 1924). The male flowers supposedly have a single whorl of four tepals, but Baillon (1877, 1887) and Hallock (1930) present evidence that in young flowers a rudimentary outer whorl alternating with the tepals exists. That this interpretation of the perianth may be incorrect will be discussed later.

The third position accorded to the Garryaceae is one suggestive of celastrinean affinity. Pulle (1952) recently revised his earlier opinion (1937) and indicated affinity with the Celastrales. Gundersen (1950) offered a fourth indirect relation with the Rubiales by placing the Umbellales (including the Garryaceae) within a supraordinal taxon, the Rubiflorae. Wangerin (1910) has indicated a similar extraordinal relation of the Cornaceae, and hence *Garrya*, with the Caprifoliaceae of the Rubiales.

Finally, Hutchinson (1926) derived nearly all amentiferous taxa from the Hamamelidaceae. The various amentiferous families with greatly reduced numbers of floral parts, syncarpy, unisexual flowers, frequent dioecism, and bracteate inflorescences seem easily derived from the Hamamelidaceae where these tendencies for reduction, syncarpy, etc., are incipient or already strongly developed. Hutchinson derived the Garryaceae from Hamamelidaceae by further reduction. Skottsberg (1940) proposed a similar position for the Garryaceae.

There remains to consider critically these suggested relationships for the Garryaceae. There will follow a comparative study based upon all available knowledge bearing upon the problem, as assembled by the senior author. Whenever the evidence under consideration has some bearing upon the greater problem of angiospermous origin, the discussion will be broadened to include it.

Comparative Anatomy of the Secondary Xylem — Table 1 represents a comparison of the secondary xylic characters of the families dealt with in this paper. With the exception of the Garryaceae, the data upon which the table is constructed were compiled from wood descriptions in various works. Original observations of certain genera have been made when feasible. Woods of the Cornaceae were examined in detail. The characters presented are those of phylogenetic and taxonomic significance. The " Anatomy of the Dicotyledons " (Metcalfe & Chalk, 1950) was consulted for all taxa. In addition, the other sources of descriptive material for each family are listed below:

Salicales — Salicaceae: Brown, Panshin & Forsaith, 1949; Record, 1934a.

Myricales—Myricaceae: Record, 1934a; Record & Hess, 1943.

Leitneriales — Leitneriaceae: Record, 1934a; Record & Hess, 1943.

Juglandales — Juglandaceae: Brown, Panshin & Forsaith, 1949; Record, 1934a; Tippo, 1938.

Piperales — Chloranthaceae: Record & Hess, 1943; Stern, 1954.

Piperales — Piperaceae: Record & Hess, 1943.

TABLE 1 — COMPARATIVE XYLIC ANATOMY

	"Amentiferae" Salicaceae	Myricaceae	Leitneriaceae	Juglandaceae	Chloranthaceae	Piperaceae	Betulaceae	Fagaceae	Ulmaceae	Moraceae	Celastrales Cyrillaceae	Aquifoliaceae	Celastraceae	Rhamnales Rhamnaceae	Umbelliflorae Araliaceae	Nyssaceae	Cornaceae	Alangiaceae I	Alangiaceae II	Rubiales Caprifoliaceae	Rosales Hamamelidaceae
Imperforate Elements																					
Types	H	+	H	+	H	H	+	H	H	H	+	+	H	H	H	+	H	H	H	H	+
Length	L	L	o	L	L	+	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Spiral thickenings present	o	o	o	o	o	o	o	o	o	o	o	+	+	+	o	o	+	o	o	+	+
Vessels																					
Porosity	+	L	L	+	L	L	L	+	+	+	L	+	L	+	+	L	L	o	o	+	L
Pore distribution	H	H	L	+	+	+	H	+	+	+	L	H	+	H	H	+	+	H	H	+	+
Diameter of elements	L	L	L	L	L	L	L	L	L	L	+	+	+	L	L	L	L	L	L	L	L
Type of perforation plate	H	+	H	H	+	H	+	H	H	H	+	+	+	H	H	+	+	+	L	+	L
Number of bars in scalariform perforation plates	o	+	H	+	L	+	+	H	H	H	L	L	+	H	+	L	L	+	H	L	L
Type of intervascular pitting	H	+	+	H	L	H	H	+	+	H	+	+	H	H	+	+	+	H	H	H	L
Type of ray-vessel pitting	H	+	L	H	L	H	H	+	+	H	+	+	H	H	L	+	+	H	H	H	L
Ray-vessel pits bordered or simple	H	+	H	H	o	o	H	H	H	+	+	+	+	+	H	+	+	o	o	H	L
Length of elements	+	L	o	L	L	H	+	+	H	H	L	L	L	+	L	L	L	L	o	L	L
Spiral thickenings present	o	L	+	+	L	L	+	+	+	+	L	+	+	+	+	+	+	o	o	L	L
Vascular Rays																					
Heterogeneous types	H	+	H	L	L	o	+	H	L	H	+	L	H	H	+	L	L	L	+	L	H
Homogeneous types	H	+	+	H	+	+	H	H	L	H	+	+	H	H	H	+	+	+	+	+	+
Sheath cells commonly present	o	+	o	o	+	o	o	o	o	o	o	+	o	o	+	o	+	o	o	+	o
Xylem Parenchyma																					
Type	o	L	o	L	L	L	L	L	+	+	L	L	L	L	L	L	L	L	L	L	L
Number of cells in strand	o	L	L	o	+	+	L	L	+	+	L	L	L	L	L	L	L	L	L	L	L
Parenchyma distribution	L	+	H	H	+	H	+	L	H	+	+	+	H	H	H	L	L	L	H	+	L
Storied Structure	+	+	+	+	+	H	+	+	H	H	+	+	+	H	+	+	+	+	+	+	+
Number of characters identical to those of the Garryaceae	3	11	4	6	7	5	8	7	7	7	10	12	8	5	7	9	10	4	4	9	8
Percentage of characters identical to those of the Garryaceae	15%	55%	20%	30%	35%	25%	40%	35%	35%	35%	50%	60%	40%	25%	35%	45%	50%	20%	20%	45%	40%
Number of characters of a lower phyletic level than those of the Garryaceae	3	7	5	5	10	4	5	5	3	2	8	7	5	4	6	9	9	7	4	7	10
Number of characters of a higher phyletic level than those of the Garryaceae	8	1	6	6	0	7	5	6	8	9	0	1	6	10	6	0	1	4	7	4	1

\+ Family in question has essentially the same secondary xylic feature as found in the Garryaceae.
H Character of the family in question is more specialized than that of the Garryaceae.
L Character of the family in question is less specialized than that of the Garryaceae.
o Knowledge of character lacking, or phylogenetic nature not understood.

The statements of lengths or diameters represent average ranges of measurements, rather than maximal ranges.

Fagales — Betulaceae: Brown, Panshin & Forsaith, 1949; Record, 1934a; Tippo, 1938.
Fagales — Fagaceae: Brown, Panshin & Forsaith, 1949; Record, 1934a; Tippo, 1938.
Urticales — Ulmaceae: Brown, Panshin & Forsaith, 1949; Record, 1934a; Tippo, 1938.
Urticales — Moraceae: Record, 1934a; Tippo, 1938.
Celastrales—Cyrillaceae: Record, 1934a; Record & Hess, 1943.
Celastrales — Aquifoliaceae: Brown, Panshin & Forsaith, 1949; Record, 1934a; Record & Hess, 1943.
Celastrales — Celastraceae: Record, 1934a; Record & Hess, 1943.
Rhamnales — Rhamnaceae: Record, 1934a.
Umbelliflorae — Araliaceae: Adams, 1949; Li & Chao, 1954.
Umbelliflorae — Nyssaceae: Adams, 1949; Li & Chao, 1954; Titman, 1949.
Umbelliflorae — Cornaceae: Adams, 1949; Li & Chao, 1954.
Umbelliflorae — Alangiaceae: Adams, 1949; Li & Chao, 1954.
Rubiales — Caprifoliaceae: Record, 1934a; Record & Hess, 1943.
Rosales — Hamamelidaceae: Tippo, 1938.

A detailed chart listing all xylic characters for all the families concerned formed the basis of Table 1 which shows concisely only the essential similarities and differences among the wood characters of the families. For details of any of them, references must be made to the descriptions cited.

Evaluation of the evidence presented in Table 1 must be based upon certain premises. The xylic characters utilized serve a two-fold purpose. In any given taxon they indicate level of specialization. They also have value in determining relationship between two taxa. A large number of important secondary xylic features of about the same degree of specialization common to Garryaceae and another family is probably indicative of a close relationship between the two if this finding is substantiated by evidence from other botanical disciplines. Likewise, if a family presents several xylic features which are similar to those of the Garryaceae and several which are a little more or less specialized, the probability of a recent common ancestor is also indicated if corroborative evidence is found from other botanical studies. On the other hand, a family having very few xylic characters of the same degree of specialization in common with the Garryaceae, and a large number of features which are a great deal more highly specialized, little or no relationship is possible. Since the major trends of structural specialization in the xylem are irreversible (Bailey, 1944a), relationship between the Garryaceae and another family which is much more specialized in xylic as well as other features is unlikely or very distant.

From Table 1 it may be observed that of the Amentiferae, the Myricaceae possess the largest number of xylic structures in common with the Garryaceae. Eleven of the 20 characters (55 per cent) are essentially similar. Seven of the remaining characters of the secondary xylem of the Myricaceae are lower in phylogenetic level than corresponding ones of the Garryaceae. Certainly, this comparative evidence suggests that the possibility of relationship should be considered further.

On the basis of wood anatomy (Table 1), the following families among the Amentiferae may be considered as quite distantly related to the Garryaceae, if at all: Salicaceae, Juglandaceae, Chloranthaceae, Piperaceae, Betulaceae. The Salicaceae, Juglandaceae, and Piperaceae show few similar wood characters and those not similar are generally more specialized. The Salicaceae are very interesting in this respect. The woods are, perhaps, more specialized than any other family of the Amentiferae and the least similar to the wood of the Garryaceae; yet, the Garryaceae are frequently listed immediately following the Salicaceae (Engler & Diels, 1936; Lawrence, 1951; Rendle, 1925; Pulle, 1937). Although proximity of two families in a classification does not necessarily connote genetic relationship, this is frequently implied. The Chloranthaceae have woods which are far more primitive, and show very few similar characters (7 or 35 per cent)

to the woods of the Garryaceae. Close relationship is not likely.

The Fagaceae, Ulmaceae, and Moraceae demonstrate only 7 (35 per cent) similarities and the Betulaceae only 8 (50 per cent) of the 20 characters studied. Relationship is improbable or very distant since the characters differing from those of the Garryaceae are generally more highly specialized. Information on the Leitneriaceae is incomplete. However, 15 of the 20 characters chosen for study are known. The wood resembles that of Garryaceae in only 4 features. Of the remaining, 6 are more highly specialized and 5 less highly specialized. This indicates a similar level of specialization, but not closeness of relation.

Comparisons have been completed between Garryaceae and the common woody families of the Celastrales: Cyrillaceae, Aquifoliaceae, and Celastraceae. Because the limits of the Celastrales are often extended to include the Rhamnaceae and allies, this family will also be discussed. Of the four families, the greatest number of characters similar to those of the Garryaceae are found in Aquifoliaceae. In the two families, the woods are similar in 12 of the 20 (60 per cent) characters studied. On the basis of wood anatomy, it would not be illogical to consider the possibility of a celatrinean ancestor for the Garryaceae.

It is not likely that the Garryaceae, as apparent from wood anatomy, are at all close to the Rhamnaceae. The woods of the latter family, although quite variable, are generally of a more highly specialized nature than those of the Garryaceae. The woods of the Cyrillaceae and Celastraceae are more similar to the wood of *Garrya* than those of Rhamnaceae.

Of the Umbelliflorae complex, the woods of the Garryaceae and Cornaceae are similar in 10 of the 20 characters studied (50 per cent). In the Cornaceae, the remaining characters except one are more primitive. The Nyssaceae are slightly less similar with 9 characters in common with those of the Garryaceae. Alone, this would be poor evidence, but the Nyssaceae and Cornaceae combined as recommended by Adams (1949), Dermen (1932), Hallier (1912), and Li & Chao (1954), demonstrate 11 (55 per cent) characters identical with those of the Garryaceae. From Table 1, it may be noted that the Araliaceae and Alangiaceae show few suggestions of relationship. If an affinity between Garryaceae and Cornaceae becomes probable, relation to the Araliaceae and Alangiaceae, although distant, would be implied.

The Caprifoliaceae, although a sympetalous taxon, have as many (9) similar wood features to the Garryaceae as do the Nyssaceae. Since extraordinal affinity of Caprifoliaceae with the Umbelliflorae has been proposed by Gundersen (1950), Wagnerin (1910), and others, some further consideration will be given to this suggested link.

The Hamamelidceae, as partially reduced derivatives of a rosaceous ancestor, have commonly been considered in recent years as a link to many more highly reduced families (e.g. Hutchinson, 1926; Moseley, 1948; Pulle, 1937; Tippo, 1938). Relation of the Garryaceae to the Hamamelidaceae, in so far as anatomical features of the xylem are concerned, is certainly not indicated. Only 8 characters (40 per cent) of the woods of the Hamamelidaceae have reached the same approximate level found in the woods of the Garryaceae. As Hutchinson (1926) has linked the Garryaceae here, as a derivative of a hamamelidaceous ancestor, it will be necessary to consider this possibility of affinity further.

In summary, the comparative wood anatomy of the Garryaceae with that of other families often linked to the Garryaceae reveals the possibilities of close relation to the Myricaceae of the amentiferous families, the Aquifoliaceae of the Celastrales, and the Cornaceae-Nyssaceae taxa of the Umbelliflorae. Affinity of the Garryaceae to either the Caprifoliaceae or the Hamamelidaceae seems remote, but further investigation will be made of these suggested links. Other families of the Amentiferae seem improbable relatives of the Garryaceae, but these families will be discussed in the light of evidence other than wood anatomy.

Before concluding this section, the relative phylogenetic level of the Garryaceae, aside from possible affinities, as

suggested by the study of the secondary xylem, should be discussed. The following characters of the secondary xylem of the Garryaceae are strictly primitive ones:

(1) The presence of tracheids and fibre-tracheids, but lack of libriform wood fibres (Bailey, 1936, 1953; Bailey & Tupper, 1918; Reinders, 1935);

(2) lack of septate imperforate elements (Metcalfe & Chalk, 1950; Tippo, 1938);

(3) numerous to very numerous vessels per square mm. (Frost, 1930a);

(4) vessel elements typically angular (Frost, 1930a);

(5) vessel elements very small in diameter (Frost, 1930a);

(6) vessel elements with scalariform perforation plates (Bailey, 1944a; Bailey & Tupper, 1918; Frost, 1930a, 1930b);

(7) vessel elements with quite oblique end walls (Bailey, 1944a; Frost, 1930a);

(8) ray vessel pitting bordered (Metcalfe & Chalk, 1950); and

(9) presence of uniseriate and multiseriate rays (Barghoorn, 1940, 1941a; Kribs, 1935).

The following characters of the secondary xylem of *Garrya* are moderately specialized:

(1) Imperforate elements most frequently have thin to thick walls (intermediate categories) (Bailey, 1936, 1953; Reinders, 1953);

(2) vessels (Frost, 1931) and (3) imperforate elements typically with circular bordered pit-pairs having crossed inner apertures which do not extend beyond the border outlines (Bailey, 1936; Eames & MacDaniels, 1947);

(4) lengths of imperforate elements ranging most frequently from 500 μ (very short) to 900 μ (moderately short) (Bailey, 1953; Bailey & Tupper, 1918; Chattaway, 1936);

(5) vessel distribution predominantly solitary but with clusters, multiples, and chains also occurring (Tippo, 1946);

(6) diffuse porosity to ring porosity, ring porosity predominant (Frost, 1930a; Gilbert, 1940);

(7) the more common occurrence of intermediate numbers of bars composing the multiple perforations (Frost, 1930b);

(8) the common presence of wide (Frost, 1930b) and (9) non-bordered perforations in the scalariform perforation plates (Frost, 1930b);

(10) opposite as well as alternate intervascular pitting (Bailey & Tupper, 1918; Frost, 1931);

(11) similar as well as transitional pitting between rays and vessels (Bailey & Tupper, 1918; Frost, 1931);

(12) length of vessel elements most frequently medium-sized (Bailey, 1953; Bailey & Tupper, 1918);

(13) Heterogeneous ray Types IIA and IIB (Barghoorn, 1941a, 1941b; Kribs, 1935);

(14) presence of strand parenchyma most commonly composed of 2 to 6 cells accompanied with true fusiform parenchyma (Metcalfe & Chalk, 1950); and

(15) diffuse and diffuse-in-aggregate distribution of wood parenchyma accompanied by the less commonly occurring banded apotracheal and scanty paratracheal types (Kribs, 1937; Metcalfe & Chalk, 1950).

Two features of the secondary xylem are highly specialized:

(1) occurrence of spiral thickenings in few to all of the imperforate elements (Tippo, 1946);

(2) spiral thickenings present in all vessel elements (Frost, 1931).

The preceding analysis signifies that the secondary xylem of *Garrya* cannot be considered primitive. Although several characters quite definitely are of a non-specialized nature, a greater number are moderately specialized and a few are highly specialized. In view of this evidence, one must tentatively conclude that the Garryaceae have reached a moderate level of specialization. Such a conclusion carries one beyond the problem of relating the Garryaceae to one taxon or another to the problem of the origin of the Angiospermae. The secondary xylary evidence alone, while it cannot invalidate entire systems of classifications or hypotheses for the origin of angiosperms, certainly requires a more objective analysis of the evidence supporting one theory or another. Phylogenetic studies based upon the secondary wood anatomy correlated with other available evidence from other fields

TABLE 2 — COMPARISON WITH TAXONOMIC CHARACTERS

	Garryaceae	"Amentiferae" Salicaceae	Myricaceae	Leitneriaceae	Juglandaceae	Chloranthaceae	Piperaceae	Betulaceae	Fagaceae	Ulmaceae	Moraceae	Celastrales Cyrillaceae	Aquifoliaceae	Celastraceae	Rhamnales Rhamnaceae	Umbelliflorae Araliaceae	Nyssaceae	Cornaceae	Alangiaceae	Rubiales Caprifoliaceae	Rosales Hamamelidaceae
1. Leaves opposite (+), or some members with leaves opposite (±), different (−)	+	±	−	−	±	+	±	−	−	−	±	−	−	±	±	±	−	±	−	+	−
2. Leaves estipulate (+), or some members with leaves estipulate (±), stipulate (−)	+	−	+	+	+	−	±	−	−	−	−	+	±	±	±	±	+	±	+	±	−
3. Simple inflorescences or units of compound inflorescences are racemes (+); spikes or some members with spikes or racemes (±); different (−)	+	±	±	−	−	±	±	−	±	−	−	+	−	−	−	±	±	±	−	−	−
4. Flowers unisexual (+), flowers of some members unisexual (±), flowers bisexual (−)	+	+	±	+	+	±	±	+	±	±	+	−	±	±	±	±	±	±	−	−	±
5. Plants dioecious (+), some members polygamodioecious or dioecious (±), plants monoecious and/or hermaphroditic (−)	+	+	±	+	±	−	−	−	±	−	±	−	±	±	−	±	±	±	−	−	±
6. Flowers achlamydeous (+), typically monochlamydeous (±), typically dichlamydeous (−) — Female	±	+	+	+	±	+	+	±	±	−	−		−	−	−	−	−	−			−
6. Flowers achlamydeous (+), typically monochlamydeous (±), typically dichlamydeous (−) — Male	+	+	+	±	±	+	+	±	±	−	−		−	−	−	−	−	−			−
6. Flowers achlamydeous (+), typically monochlamydeous (±), typically dichlamydeous (−) — Bisexual			+			+	+			−		−	−	−	−	−		−	−	−	−
7. Perianth essentially tetramerous in all or most members (+), different (−)	+	−	−	+	+	−	−	+	+	+	+	−	+	+	−	−	+	+	+	−	+
8. Tendency toward future reduction of perianth parts indicated (+), no indications of future reduction (−)	+	−	−	−	+	−	−	+	−	−	−	−	−	+	+	+	+	+	+	−	+
9. Apparently polypetalous or formerly so (+), tendency to sympetaly (−)	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	+	+	−	+
10. Anther basifixed (+), anther different (−)	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	+	+	+	−	+
11. Ovary superior (+), ovary inferior (−)	+	+	+	+	−	−	+	−	−	+	±	+	+	+	+	−	−	−	−	−	−

12. Carpels 2 (+), 2 or more carpels (±), different (−). (All gynoecia probably syncarpous.)	+	±	+	−	±	−	±	+	±	+	+	±	−	±	±	−	±	±	±	−	+
13. Locules of syncarpous ovary 1 (+), locules 1 in some members (±), locules always more than 1 (−)	+	+	+	+	−	+	+	±	−	+	±	−	−	−	−	−	−	±	±	±	−
14. Placentation basically parietal (+), axile with tendency to parietal (±), other (−)	+	+	−	+	−	−	−	−	−	+	+	−	−	−	−	−	−	±	−	−	−
15. Intrastaminal disc absent (+), present (−)	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+
16. Endosperm copious or moderately abundant (+), scanty or absent (−)	+	−	−	−	−	+	+	−	−	−	±	+	+	+	+	+	−	+	+	+	−
17. Embryo small (+), embryo large (−)	+	−	−	−	−	+	+	−	−	−	−	+	+	+	−	+	+	+	+	+	−
18. Integument 1 (+), integuments 2 (−)	+	−	+	−	+	−	−	+	+	−	−	−	+	O	−	+	+	+	+	+	−
19. Embryo straight (+), embryo different (−)	+	+	+	+	+	+	+	+	+	±	−	+	+	+	+	+	+	+	+	+	+
20. Inflorescences amentiferous (+), not amentiferous (−). (Not included in totals below.)	+	+	+	+	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−
21. Number of characters identical to those of the Garryaceae	19	11	11	12	10	9	8	10	7	7	6	7	7	6	5	5	7	8	9	6	7
22. Number and percentage of characters identical to those of the Garryaceae in at least some members		14	14	13	14	11	13	12	12	9	11	8	10	11	9	10	12	16	11	8	9
		74%	74%	68%	74%	58%	68%	63%	63%	47%	58%	42%	53%	58%	47%	53%	63%	84%	58%	42%	47%

\+ Characters identical to those of the Garryaceae
− Characters different from those of the Garryaceae
± Characters identical at least in some members to those of the Garryaceae
O Character unknown or not understood

have been published on the Moraceae (Tippo, 1938), Juglandaceae (Heimsch & Wetmore, 1939), Fagaceae (Tippo, 1938), Rhoipteleaceae (Withner, 1941), and Casuarinaceae (Moseley, 1948). In each of these studies, the consideration of all evidence has precluded the retention of the family in question as a primitive one. Since assignment of primitive status to these families forms the corner stones of the systems of Engler (1897), Engler & Diels (1936), Rendle (1925), and Wettstein (1935), such evidence suggests that an objective, critical reconsideration of the Englerian and " Neo-Englerian " systems is needed. Such studies which utilize the "synthetic", inclusive approach also question the opposing opinions based upon work which, although thorough, is of a more restricted, exclusive scope (Fagerlind, 1946; Hagerup, 1934, 1936, 1938; Hjelmqvist, 1948; Lam, 1948, 1952).

A Correlation of Taxonomic Features with Xylem Characters — Table 2 has been prepared in a manner to show differences and similarities of taxonomic features between the Garryaceae and each of the families to which the Garryaceae have been linked. As familial taxonomic descriptions are readily available, they are not included here. The characters chosen for comparison are those for which information is available in standard taxonomic works. Item 21 in Table 2 represents for each family the number of characters which are essentially similar in all members with those of the Garryaceae. The similarities are obviously very few in any comparison. Item 22, however, represents for each family the number of characters which are essentially identical to those of the Garryaceae in all members or at least some members of each family. More conclusive results are demonstrable in this fashion. The inclusion of similar characters appearing in only some members of a family may be defended by calling attention to the fact that but a single genus, *Garrya*, is being compared to families with two to several genera (except the Leitneriaceae).

Table 2 reveals the chief cause of the difficulty of assigning the Garryaceae to a natural position and why so many systematists have placed the family with the Amentiferae. The amentiferous families all have bracteate inflorescences, most have unisexual flowers and are dioecious or have at least some members so, and nearly all are achlamydeous or monochlamydeous. These characters, present in the Garryaceae as well, are those which seem to unite the many families of the Amentiferae. These exomorphic features coupled with certain anomalous embryological features (chalazogamy, multicellular archesporium), often said to have been retained from coniferous ancestors, have caused some authors to conclude that all are essentially primitive (Engler & Diels, 1936; Hjelmqvist, 1948, 1953; Rendle, 1925; Wettstein, 1935).

The comparison of taxonomic characters in Table 2 reveals the possibility of a basic fallacy, that of concluding that the similarity of a few striking features, usually exomorphic, indicates close affinity. Many definitions of the term ament (or catkin) indicate only that it is a bracteate inflorescence. Other definitions state that a catkin is a bracteate spike or spike-like inflorescence of cymules. The inflorescence of *Garrya* is essentially a simple or compound raceme and is an ament only because of its bracteate nature. Not a single one of the woody amentiferous families has inflorescences which are essentially racemose. Five amentiferous families, however, have inflorescences which are basically spikes and hence structurally similar to that of *Garrya*. Other amentiferous families have compound inflorescences composed of units of cymules, panicles, cymes, or heads. The presence of an ament can hardly be considered an indication of relationship, merely a modification of some other type associated with anemophily. Because of this lack of real structural similarity among aments of the different families, the presence of aments has not been considered of taxonomic importance and has not been used in totaling similarities and differences. The true nature of the inflorescence has been considered, however. Unisexuality, dioecism, and monochlamydy or achlamydy in these families may also be the result of parallel evolution. Since these

characters appear in families other than amentaceous ones, they cannot be ignored.

Stebbins (1950) has pointed out that the relative simplicity of plant structures, compared to those of animals, limits the degree of specialization in a given direction. This simplicity favors a large amount of parallel evolution and hence morphological similarity in plants is less indicative of close relationship than in animals. Stebbins (1951) accepts the probability that certain combinations of characters appear more frequently because they have adaptive value and he points to the amentaceous groups as examples. In view of the low degree of similarity of xylic features between Garryaceae and any one of the amentiferous taxa, Myricaceae excepted, and relatively higher proportion of both xylic and taxonomic features (Tables 1 and 2) between the Garryaceae and Cornaceae affinity with all but the Myricaceae of the Amentiferae is again precluded.

The possibility of close relation of Garryaceae to any family of the Celastrales seems ruled out by the relatively low number of similarities, generally lower than in the amentiferous families. The tendencies toward unisexuality, dioecism, and floral reduction have perhaps inspired the idea that Garryaceae may have been derived by reduction from a celastrinean ancestor. The 42-58 per cent occurrence of similar taxonomic characters accompanied with the similarity of the woods of Aquifoliaceae and Garryaceae, perhaps, suggest a distant extraordinal affinity.

A relation of the Garryaceae to the Rhamnaceae seems highly improbable on taxonomic as well as anatomical grounds. The proportions of similarities of wood characters is very low, and of taxonomic characters only moderately high.

The correlation of similarities found in the Garryaceae and in at least some members of Cornaceae is high (84 per cent) and indicative of possible relationship. The racemose inflorescence, the basic type found in Garryaceae, occurs in a few of the Cornaceae. Opposite and estipulate leaves, characteristic of the Garryaceae, occur in a few members of the Cornaceae. Although no aments occur in Cornaceae, unisexual flowers are found as well as bisexual ones, and of the members with unisexual flowers, dioecism or polygamodioecism is common. The gynoecium of the Garryaceae is syncarpous, bicarpellate, and unilocular. The same condition appears in some Cornaceae. The basically parietal placentation found in *Garrya* is also found in *Aucuba* of the Cornaceae. The parietal placentation of both of these genera may have been derived from axile placentation (Eames & MacDaniels, 1947) found commonly in Cornaceae, Nyssaceae, and Araliaceae. In the Garryaceae and Cornaceae the embryo is small and surrounded by copious endosperm, and the ovules have but a single integument.

The ovary of *Garrya* has been described as inferior by Baillon (1877, 1887) and by Hallock (1930) because of the observance of rudimentary epigynous perianth parts. Most taxonomic descriptions have overlooked or failed to discuss these studies. According to Eames (1929) and Eames & MacDaniels (1947), floral rudiments with vascular tissue are usually vestigial rather than incipient perianth parts. An epigynous gynoecium would eliminate one of the chief reasons for placing *Garrya* among the amentiferous taxa, and support the inclusion of *Garrya* among the epigynous Umbelliflorae. Apparently, epigyny has arisen in the other taxa of the Umbelliflorae by adnation of the perianth to the ovary (Lawrence, 1951). In the Garryaceae, however, according to Reeve (1943), the double rudimentary perianth of the female flowers and outer rudimentary perianth whorl of the male flowers described in *G. elliptica* by Hallock (1930) are really reduced bracts which do not develop beyond the prophyll stage and hence are homologous to the bracts at the base of the less reduced secondary portions of the branched inflorescences of *G. laurifolia*, *G. gracilis*, and other more tropical species. The fact that the structures occur in decussate pairs on the ovary does not support the view that they are reduced bracts of the more primitive and branched inflorescences. For example, each female flower in *G. laurifolia* is subtended by a single

bract not a decussate pair. The matter needs further clarification before deviating from generally held opinions. There is a strong tendency for the reduction of floral parts in Cornaceae, followed by adnation of perianth parts to the ovary. The achlamydy and monochlamydy of the Garryaceae may be the result of reduction initiated, however, in the ancestral stocks of the two families previous to adnation and epigyny.

Many of the tendencies seen now in some members of the Cornaceae, which probably arose in ancestral stock, could have produced the extreme conditions of *Garrya* further modified by the amentiferous structure and anemophily of the genus. The similarity of the woods of *Garrya* and Cornaceae have also been presented. It was further mentioned that the dissimilarities of xylic features were represented by less specialized conditions in the Cornaceae. The possibility of derivation of *Garrya* from some ancestral stock common to that of the Cornaceae is a distinct possibility.

Gundersen (1950) included the Umbellales with the Rubiales within a supraordinal taxon termed the Rubiflorae. This reflects the views of others (Coulter & Chamberlain, 1903; Wagnerin, 1910; Wilkinson, 1949) who believe the Caprifoliaceae and Cornaceae are closely related. The resemblance of *Cornus* to *Viburnum* and *Sambucus* is frequently noted. The Garryaceae, on the other hand, are not particularly close to the Caprifoliaceae. The woods of the Caprifoliaceae are slightly more specialized along different lines than the Garryaceae. From Table 2 it can be seen that the Garryaceae and Caprifoliaceae are not closely similar in taxonomic features. It is thought that the two families probably stand on separate evolutionary lines which arose from a pro-umbelliflorean ancestor with strong cornaceous characters.

It has already been stressed that the derivatives of the Garryaceae from a hamamelidaceous ancestor is not indicated by the comparison of the secondary xylems of the two taxa. From Table 2 it can be observed that the Hamamelidaceae resemble the Garryaceae in only 7 of the 19 taxonomic characters studied; and, that if partial similarity is invoked (some member resemblance), similarity in only 9 characters (47 per cent) obtains.

The strong tendencies to reduction of floral parts, coupled with the primitive secondary xylem, have made this family a ready link for Amentiferae to the rosalian complex (Hutchinson, 1926; Moseley, 1948; Tippo, 1938). In short, many highly reduced, especially amentaceous taxa, have been attached here. This " link " has been unduly utilized. It does not seem probable that all of the achlamydeous or monochlamydeous, amentiferous families arose from a hamamelidaceous ancestor.

In summary, the comparative study of taxonomic characters and a correlation of these with results from the comparative study of the secondary xylem supports the tentative proposal that the Garryaceae are derivatives of a pro-umbelliflorean stock, cornaceous in nature, which gave rise also to the Cornaceae and Nyssaceae. The Caprifoliaceae may represent derivatives of a third type from the same generalized ancestor, and they are now more closely related to the Cornaceae than to the Garryaceae. The possibility of a close link to any of the Celastrales, suggested by similarities in the woods, is not confirmed because of a low degree of similarity of taxonomic characters. Similarly, the likelihood of a derivation of the Garryaceae from the Hamamelidaceae, at all directly, seems precluded by a lack of similarities. Finally, the degree of similarity between Myricaceae and Garryaceae is, if anything, increased by the comparison of taxonomic features.

Phloem Anatomy — Information on the comparative anatomy of the secondary phloem of the dicotyledons is relatively meager. The previous description of the phloem cannot be utilized, therefore, in ascertaining the taxonomic position of the Garryaceae. In general, however, the degree of specialization is moderately high. Although the elongate sieve-tube elements with compound sieve plates on oblique end-walls are fairly low in angiospermous evolutionary specialization (Esau, 1953; Esau, Cheadle & Gifford, 1953), the regular, sequential arrangement of cell types into alternating

tangential bands of fibres and living cells, and the occurrence of large, flaring multiseriate rays which intersect the other cells are indications of moderate specialization. The frequent occurrence of sclerotic ray cells and of a high proportion of phloem fibres often accompanies specialized xeromorphic features. The structure of the secondary phloem lends support to the thesis that the Garryaceae are a moderately specialized taxon and not a primitive one.

Nodal Anatomy—Nodal anatomy has been used for some time as an aid in systematic problems. It has been suggested (Marsden & Bailey, 1955) that the double-trace, unilacunar node is the primitive type and the trilacunar and some unilacunar types of Sinnott (1914) derived, and hence, moderately advanced. The multilacunar and many unilacunar forms (Sinnott, 1914) are thought to be most specialized, and derived from the trilacunar.

Table 3 represents the nodal conditions of the families discussed in this paper. The number of gaps are taken from the listing by Sinnott (1914), and the trilacunar condition of *Garrya* was verified by the examination of nodal regions of seven species. It may be noted that most of the amentiferous taxa, the Garryaceae, and the Rhamnaceae have the trilacunar, moderately advanced type; however, these taxa are sufficiently different in the respects previously discussed that indication of relationship seems unjustified regardless of this character. That the Cornaceae and Nyssaceae are trilacunar is, in view of txonomic and wood resemblances, correspondingly positive evidence supporting the proposed relationship. The trilacunar condition of the Hamamelidaceae is not sufficient, in light of the many differences in wood and taxonomic features, to support derivation of the Garryaceae from a hamamelidaceous ancestor.

The tendency for the unilacunar nodal condition (probably derived from the trilacunar) to appear in the Aquifoliaceae, and its constant occurrence in the Cyrillaceae and the Celastraceae, does not sustain the proposal that the Garryaceae are celastrinean in affinity (Pulle, 1952). The occurrence of the unilacunar node in the Myricaceae, although trilacunar nodes predominate, is a challenge to the affinity suggested by taxonomic and xylic resemblances. The multilacunate condition in the Juglandaceae, Chloranthaceae, Piperaceae, and Moraceae support the assumption of remote relationship of these to the Garryaceae. The multilacunate condition of the Araliaceae and Caprifoliaceae support the thesis that these families represent dichlamydeous taxa not closely related to the Garryaceae.

Floral Anatomy — As the floral anatomy of the Garryaceae has not been completely studied, there is from this source no direct contribution to the taxonomic problem of the family. Indirectly, with reference to the proposed relation of the Cornaceae and Caprifoliaceae, it is pertinent to note the investigations of Wilkinson (1949). She concluded that the comparative evidence (Horne, 1914; Wilkinson, 1944) from floral anatomy indicates the possible derivation of the *Viburnum-Sambucus* group from primitive Cornaceae. This evidence supports the xylic evidence for the derived

TABLE 3

Nodes Trilacunar

Garryaceae	Rhamnaceae
Salicaceae	Nyssaceae
Leitneriaceae	Cornaceae
Betulaceae	Alangiaceae
Fagaceae	Hamamelidaceae
Ulmaceae	

Nodes Trilacunar or Unilacunar — Single Trace

Myricaceae	Aquifoliaceae

Nodes Unilacunar — Single Trace

Cyrillaceae	Celastraceae

Nodes Trilacunar or Multilacunar

Juglandaceae	Moraceae
Chloranthaceae	Araliaceae
Piperaceae	Caprifoliaceae

Nodal conditions of the Garryaceae and supposed allies.

position of the Caprifoliaceae and hence the lack of a direct link between the Caprifoliaceae and *Garrya*.

The inflorescence and floral anatomies of many amentiferous families have been investigated: Salicaceae (Fisher, 1928); Leitneriaceae (Abbe & Earle, 1940); Juglandaceae (Langdon, 1939; Manning, 1938, 1940, 1948); Betulaceae (Abbe, 1935); Fagaceae (Langdon, 1939; Berridge, 1914); Ulmaceae, Moraceae, and Urticaceae (Bechtel, 1921). In every investigated case, the authors decided that the superficially simple inflorescences were the result of great condensation, reduction, and loss of parts from complex types. The flowers demonstrated sound anatomical evidence in the presence of vestigial traces (Eames, 1929; Eames & MacDaniels, 1947) indicating the reduction and loss of perianth parts, the reduction of carpel number, and, where unisexuality exists, a former hermaphroditism. In no case could the inflorescences and flowers be interpreted as transitional structures between coniferous or ephedroid ancestors and angiosperms (Engler & Diels, 1936; Fagerlind, 1946; Hagerup, 1934, 1936, 1938; Hjelmqvist, 1948; Lam, 1952; Wettstein, 1935) nor could the families concerned be considered primitive. It is important to recall that the evidence from the studies of the secondary xylems of the amentiferous families (e.g. Heimsch & Wetmore, 1939; Moseley, 1948; Tippo, 1938) strongly coincide with the evidence of floral anatomy.

There is some suggestion that the inflorescences and flowers of *Garrya* are also products of reduction. In the more primitive species of the warmer portions of the distribution area (Axelrod, 1952; Camp, 1947), the inflorescences are branched with short, bracteate axillary portions. In the more temperate species, the inflorescences are condensed and imbricate. Although the female flowers are achlamydeous, the male flowers are monochlamydeous, probable evidence of the loss of parts. The presence of a rudimentary ovary in male flowers points to a probable former hermaphroditism and perfect flowers. All of these indications of reduction support to a small degree the impossibility of according a primitive status to the Garryaceae.

Pollen Morphology—In recent years, characters of the pollen grains have been used to assist in solving taxonomic problems. Certain hereditary characteristics of the pollen are of taxonomic importance, and grains of the same species and related species (Wodehouse, 1928, 1935), and quite often related genera (Erdtmann, 1943, 1952) are closely similar. For the dicotyledons, the gross number of germinal furrows is of phyletic importance. A monocolpate grain characterizes primitive groups and tricolpate grains or those with higher numbers of furrows characterize higher groups. The number of furrows in grains of multicolpate families may vary within a species depending upon the arrangement of the quartets, however. The form and nature of the furrows are chiefly hereditary and phyletic, and are generally constant throughout a family, although notable exceptions occur. The shape of the grain and the type of external sculpturing are also of value. As with many other types of evidence, the palynological features cannot be used alone because the combinations of characters may reappear in unrelated families (Wodehouse, 1928).

Table 4 presents pollen characters of the families of greater importance to the discussion. The descriptive material has been assembled from the three principal texts (Erdtmann, 1943, 1952; Wodehouse, 1935). The terminology is that found in Erdtmann (1952).

From Table 4 it may be seen that the pollen grains of the Myricaceae differ strikingly from those of *Garrya*. This is relatively strong evidence against a possible relationship between *Garrya* and the Myricaceae. The grains of the Aquifoliaceae differ in details of the germinal furrows, in shape, and in sculpturing of the grains of some species. In view of the only moderate correlation of taxonomic features and the tendency to unilacunar nodes, the palynological evidence supports the concept that *Garrya* is not related to celastrinean taxa. The grains of the Rhamnaceae are quite similar to those of *Garrya*, at least in some members. Because of the outstanding

TABLE 4

	Furrow Condition	Diameter in μ	Surface	Shape
Garryaceae	3-colporate	31-38	Reticulate to slightly retipilate	Suboblate to oblate-spheroidal
Myricaceae	3-pororate	24-38	Crassisexinous	Suboblate to sub-oblate-spheroidal
Aquifoliaceae	3-colporoidate	20-50	Reticulate, verrucate, smooth	Spheroidal prolate
Rhamnaceae	3-colporate	17-35	Reticulate	Suboblate to subprolate
Nyssaceae	3-colpate	32	Granular	Spheroidal
Cornaceae	3-colporate	15-67	Reticulate or granular	Oblate spheroidal
Caprifoliaceae	3-colpate	40	Subechinate	Spheroidal to sub-oblate
Hamamlidaceae	3-colpate	19-34	Reticulate to pitted	Spheroidal

Pollen characters of supposed allies of the Garryaceae

lack of similarities in both the secondary xylems and general taxonomic features of the two taxa, this evidence is insufficient to uphold relationship.

The grains of the Cornaceae are closely similar to those of *Garrya*. Some members, however, have granular grains and this is reflected in the Nyssaceae, supposedly related. This evidence supports the proposed relation of *Garrya* to the Cornaceae, but favours separation of *Nyssa* and *Garrya*. The grains of the Caprifoliaceae differ chiefly in surface sculpturing from both the Cornaceae and Garryaceae. This substantiates the claim from previously stated evidence that *Garrya* is but distantly related. There is a close similarity among the grains of Hamamelidaceae, Garryaceae, and Cornaceae. With the rather great differences in xylic and floral characteristics of the Garryaceae and Hamamelidaceae, the palynological features seem insufficient to bring the two taxa closer, and less significant in comparison to the similarities of pollen, wood, and taxonomic features in Cornaceae and *Garrya*.

With respect to the related problem of the general phylogenetic level of *Garrya* and the origin of the angiosperms, the Garryaceae offer evidence again of a derived rather than a primitive nature. The microspores are tricolporate, a condition considered advanced over the more primitive monocolpate type found in the gymnosperms (unquestionably more primitive) and the Monocotyledoneae.

Cytotaxonomy—Chromosome complements of related taxa often show a definite relationship and demonstrate their mode of derivation from a basic number (Stebbins, 1950; Wanscher, 1934). Rather inconclusive data among karyological studies of the critically important families concerned in this study are available (Darlington and Janaki-Ammal, 1945; Dermen, 1932; Gaiser, 1926, 1930a, 1930b; Gundersen, 1950; Tischler, 1927, 1931, 1937, 1938). In most families aneuploid alteration has been most frequent with autoploidy or amphidiploidy playing secondary parts.

The *haploid* numbers which have been recorded are:

Myricaceae — 8 and 16;
Aquifoliaceae — 9, 10, 13, 17;
Rhamnaceae — 10, 12, 13;
Cornaceae — 8, 9, 10, 11, 12, 16, 18, 22, 36, 47;
Caprifoliaceae — 8, 9, 10, 16, 18, 19, 20, 27;
Hamamelidaceae — 12, 15, 24, 36.

The only successful count of the chromosome number in *Garrya* has been

by Meurmann (1930). The haploid number for *G. elliptica* was found to be 11. This single count is hardly helpful. The count does not pose any difficulty with deriving Garryaceae from a cornaceous ancestor where aneuploidy seems to have been the principal mechanism of evolution. Neither does the evidence effectively negate affinity with the other five families except notably with the Myricaceae.

The configuration of the chromosome complement at meiosis has been only poorly seen and described for *Garrya* (Meurmann, 1930) and admits of no comparison with the configurations of other families. That karyological evidence should be considered at all in this study may be questioned. However, Stebbins (1950) points out that the differences among the higher categories have originally had a selective basis and that they arose at species level. He states further that the mechanisms of evolution involved in the differentiation of higher categories has been the same as those which gave rise to subspecies and species.

Developmental Anatomy — The developmental anatomy of both vegetative shoot and floral apices in Garryaceae was described (Reeve, 1942, 1943). Mention has already been made of the development of the prophylls in the inflorescence and the implications of their presence. One further character deserves mention. The appearance of the tunica-corpus varies with the stage of the plastrochon (sequence of developmental changes in the shoot apex from the initiation of one leaf primordium to the next), but at all times the tunica and the protoderm derived from it remain a single, discrete layer. All divisions in the tunica are anticlinal and they contribute only to the protoderm. The presence of a single tunica layer with only anticlinal divisions represents the highest development of the shoot apex of the angiosperms (Popham, 1951). The high phyletic level of the type of shoot apical development emphasizes again the thesis that *Garrya* is not primitive, but at least a moderately advanced genus.

Embryogeny — Great caution must be used in utilizing embryological data. Certain characters, such as mesogamy, chalazogamy, and multicellular archesporia occur sporadically in unrelated families (e.g. multicellular archesporia in Onagraceae, Casuarinaceae, Cornaceae, Betulaceae, Chloranthaceae) (Johansen, 1950; Maheshwari, 1950; Schnarf, 1931). Characters of this nature have questionable phyletic significance in view of the more constant gametophytic characters, such as the embryo sac type, " double fertilization ", non-motile sperms, etc. (Maheshwari, 1950), and in view of our present confused state of knowledge of these matters.

The embryo type has similarly a limited application. It may vary within a single family (Ulmaceae, Araliaceae) or between closely related families (Piperaceae, Chloranthaceae) (Johansen, 1950). If the same type is found in two families linked by similarities of other characters, then, the embryo type becomes of slight corroborative consequence. The embryo type in *Garrya* is considered to be the *Sherardia* variation of the Solanad type (Hallock, 1930; Johansen, 1950). Unfortunately, the embryo types of the critical families (Myricaceae, Aquifoliaceae, Cornaceae, Nyssaceae, and Hamamelidaceae) are unknown or insufficiently understood to be of use.

The embryo-sac is the normal, monosporic, *Polygonum* type (Hallock, 1930; Maheshwari, 1950). The normal type occurs in 70 per cent (Maheshwari, 1948, 1950) of the angiosperms and is so constant a feature that it often transcends value at familial level. The Myricaceae, Aquifoliaceae, Araliaceae, Alangiaceae, Nyssaceae, most Cornaceae, and Hamamelidaceae all possess the same normal type (Maheshwari, 1950; Mitra & Dutta, 1949; Schnarf, 1931; Shoemaker, 1905). The *Scilla* type occurs in the Celastraceae and Rhamnaceae (Maheshwari, 1950; Schnarf, 1931) and may indicate a relation between these families and a dissimilarity between the Rhamnaceae and Garryaceae.

In summary, the embryological data are so incomplete that they are of relatively little use. The evidence from embryo-sac types does not negate the proposed linkage of Garryaceae and Cornaceae. The distribution of the *Scilla* type supports

affinities between Rhamnaceae and Celastrales and the separation of Garryaceae from Rhamnaceae.

PALEOBOTANY — The evidence from paleobotany is scarcely helpful. All data have been assembled from catalogue listings and should be fairly thorough (Arnold, 1947; Darrah, 1939; Dorf, 1930; Edwards, 1931; Knowlton, 1919; LaMotte, 1944, 1952; Nagalhard, 1922; Nagel, 1915, 1916). Representatives of all families considered, except the Garryaceae and Leitneriaceae, have been found at least as far back as the Upper Cretaceous. *Garrya* has been reported as far back as Pliocene levels, and *Leitneria*, the Pleistocene.

The records for California and the southwestern United States are less complete, but all families conceivably close to the Garryaceae have been reported from older strata than has *Garrya* which goes back to the Pliocene. Myricaceae have been found in the Eocene of this area, Aquifoliaceae in the Eocene, Nyssaceae in the Eocene, Cornaceae in the Paleocene, Caprifoliaceae in the Paleocene, and Hamamelidaceae in the Eocene.

Angiosperm origin is thought to have occurred in tropical and semi-tropical, humid uplands (Axelrod, 1952; Camp, 1947; Lawrence, 1951; Stebbins, 1950), possibly in Permo-Triassic or Jurassic time, followed by the differentiation of many families or trends leading to them (Stebbins, 1950) during the Cretaceous and their spread to lowland areas. It is likely that *Garrya* is older than the Pliocene, but neither the place nor the time of origin are known, although present distribution would suggest origin in northern Mexico. The leaves of *Garrya* are similar to the leaves of several other chaparral plants (e.g. *Quercus*, *Arctostaphylos*); and since fossil identifications of many fossil genera have been by leaf remains, the lack of reports from older strata may be a matter of misinterpretation. At present, no assistance may be gained from paleobotanical reports.

ECOLOGICAL MORPHOLOGY AND ANATOMY — The leaf of *Garrya* has become specialized and presents many semi-xeric adaptations. The leaf, of the northern and Pacific Coast species especially, is thick, coriaceous, and typically covered with abundant hairs. The outer walls of the epidermis are strongly thickened. The stomata are protected by beak-like extensions of the guard or subsidiary cells and are found on only the lower surface of the leaf. There is a large-celled hypodermis and multiple palisade layers. (Faure, 1924; Metcalfe & Chalk, 1950).

The leaves are non-deciduous and evergreen, a frequent adaptation to drought in the chaparral habitat. Supposedly, the immediate availability of green leaves reduces the time that it takes the plant to produce an effective amount of food following the first rains of the short chaparral rainy season. These specialized characters are commonly found in desert and chaparral plants.

A few internal stem characters suggest an adaptation to the dry habitat. The sheath of fibrous elements at the outer border of the stele (protophloic fibres) may be an adaptation which reduces the loss of water from the stem. The cells of the larger rays often become sclerotic in the phloem adding to the sclerenchymatous sheath.

The various ecological adaptations are of such a nature that they could arise fairly quickly in primitive plants; but, associated with the many other indications of specialization, they are significant. Their presence supports the thesis of the derived, specialized nature of the Garryaceae.

PHYTOCHEMISTRY—It becomes obvious from a survey of the literature (Beilstein *et al.* 1918-1954; Heilbron & Bunbury, 1934-1938; Henry, 1949; Manske & Holmes, 1950; McNair, 1929, 1931) that many substances produced by plants are too widespread to be of use in a phylogenetic study. Some substances, on the other hand, are too rarely found to be of value. The occurrence of alkaloids can be of value and the capability of plants to produce such a compound is an indication of genetic relationship if correlated with similarities of other features. Most of the families of this study do not form alkaloids, and this discipline is of no practical help. Two questionable alkaloids have been reported for *Garrya* in

recent times (Henry, 1949), garryine ($C_{22}H_{32}O_2N.H_2O$) and veatchine ($C_{22}H_{32}O_2N$); but they have not been found in other plants.

Herissey & Lebas (1910) reported the presence of "aucubine" in three species (including *G. elliptica*) of *Garrya*. Aucubine is also present in *Aucuba* (Lebas, 1909) of the Cornaceae. Were the results more recently verified by modern methods, this evidence might be of significance. The substance, however, has not been reported in Beilstein *et al.* (1918-1954) for either *Garrya* or *Aucuba*. In Heilbron & Bunbury (1934-1938) aucubine is listed as occurring in *Aucuba japonica* Thunb. and *Plantago lanceolata* L. but not in *Garrya*. Heilbron and Bunbury term the substance a glucoside and give its empirical formula as $C_{15}H_{24}O_9$. Karyone & Kondo (1928) have verified its presence in *Aucuba*, but not in *Garrya*. One cannot justifiably utilize this evidence at this time.

Malligson (1922) completed sero-diagnostic tests with the Garryaceae and supposed related amentiferous families. The serological method does not permit the construction of a phylogenetic scheme for the entire group of angiosperms but allows only for testing a circumscribed group of related families. Malligson deals with the so-called Centrospermae. The Garryaceae are depicted as derivatives of Juglandaceae, or their ancestral group, along a limited "side-line" of affinity with Salicaceae as the ultimately derived taxon. As Juglandaceae and Salicaceae demonstrate, on the basis of nearly all other evidence, the greatest lack of similarity of any amentiferous taxa to the Garryaceae, this thesis can hardly be accepted.

Conclusions

The Taxonomic Position of the Garryaceae — A consideration of the evidence strongly favours, over all proposed alliances, a relationship of Cornaceae and Garryaceae and the conception that the Garryaceae have arisen by specialization along lines of reduction from a proumbelliflorean ancestor, cornaceous in nature. The greatest number of similarities of all sorts described occur in these two families; and, where similarities are lacking, clear, logical sequences of specialization from Cornaceae to *Garrya* are often evident.

The secondary xylems of the two families are similar in 50 per cent of the characters studied, and the wood of the Cornaceae is more primitive in nearly all remaining features. The study of taxonomic characters reveals many similarities or abundant indications of reduction in Cornaceae, tendencies which could have given rise to the Garryaceae. Similarities in details of the nodal anatomy and pollen morphology offer outstanding evidence to support the theory of alliance. Evidence from karyological studies, although meagre, offer the possibility of derivation of the chromosome number from Cornaceae as the result of aneuploidy. Embryological and paleobotanical data are inconclusive, but do not negate the assumption of derivation. Data from phytochemical studies of various sorts are of little help except to report the presence of specific alkaloids not found in other taxa. The differences between the Garryaceae and other families of the Umbelliflorae, especially the presence of amentaceous characters, is believed sufficient to warrant the separation of *Garrya* from Cornaceae in its own family, the Garryaceae.

In the previous studies, the Araliaceae and Alangiaceae have proved to be much less closely related to Garryaceae than to Cornaceae. It is assumed that these families are related only through ancient ancestral stock. The Nyssaceae have demonstrated nearly as many similarities to the Garryaceae as Cornaceae. This is expected if the Nyssaceae are part of the Cornaceae.

The study of the secondary xylems of the various amentiferous taxa indicates only one likely relationship, that with the Myricaceae. The perusal of taxonomic features reveals a moderately high number of common features between the Garryaceae and other Amentiferae, the crux of the difficulty. In view of the strikingly low correlation of similar secondary xylic characters and pollen morpho-

logical features, the higher correlation of similarities between the Garryaceae and Cornaceae, and the hypothesis that taxonomic similarities may be and can be the result of convergent evolution, relation of the Garryaceae to any of the Amentiferae except the Myricaceae seems extremely unlikely.

The relation of Garryaceae to Myricaceae, strongly suggested by similarities of wood anatomy and taxonomic characters, is negated or greatly weakened by the tendency to unilacunar nodes, the unquestionable differences in pollen types, and the lack of favourable evidence of a karyological nature.

An alliance of Garryaceae with Celastrales is supported by the relatively high correlation of similar xylic characters in the Aquifoliaceae and Garryaceae. Relation is also favoured by karyological evidence and by the presence of the normal type of embryo-sac in Aquifoliaceae. The suggested relation is more or less precluded, however, by very few similarities of taxonomic features, differences in nodal anatomy, and pollen structure.

There is relatively little evidence for an affinity between *Garrya* and Rhamnaceae. Similarities in xylic, taxonomic, and embryological characteristics are lacking or few. Only in nodal anatomy, pollen structure, and chromosome numbers is affinity suggested. Since several families possess trilacunar nodes and offer the possibility of chromosome evolution, it seems doubtful that there is enough evidence to assume any relation between *Garrya* and Rhamnaceae.

In many respects the Garryaceae and Caprifoliaceae stand apart: taxonomic characters, nodal anatomy, pollen morphology, and embryogeny. In xylic features the two taxa are moderately similar, although the Caprifoliaceae present several more specialized characters. An aneuploid relation is not impossible. The preponderance of evidence, however, seems to separate the two families.

Finally, differences among characters separate *Garrya* from Hamamelidaceae. Close similarities exist in nodal and pollen structure and a descending aneuploid origin of *Garrya* from the Hamamelidaceae is possible. The phyletic levels of the two families, as demonstrated by xylic and taxonomic features, however, are too far apart to signify derivation of the former from the latter. Possibly, the older pro-umbelliflorean ancestral stock, common point of origin for all Umbelliflorae, arose from rosaceous or hamamelidaceous archetypes. This supposition still separates Garryaceae from Hamamelidaceae and derives the Garryaceae from more closely allied cornaceous stock.

The Phylogenetic Level of the Garryaceae and Implications — Aside from the attempt to find a natural classificatory position for the Garryaceae, the phyletic level of the family and the bearing of this on the problem of the origin of the Angiospermae have been investigated. The secondary xylem was found to have several primitive characters (9), a larger number of moderately advanced characters (14), and few highly advanced features (2). Evidence was presented which implied that reduction has almost certainly occurred in the inflorescence and flowers of *Garrya*. The two-carpelled, one-loculed gynoecium with a single pendulous, parietal ovule, in view of other specialized characters, is probably a reduced condition.

The secondary phloem presented primitive sieve tube elements but several specialized features in the arrangement of cells and sclerenchymatization of rays. The nodal anatomy was examined to verify earlier investigations and found to be trilacunar, a moderately advanced dicotyledonous type. The pollen grains testify to the derived, rather than primitive level of *Garrya*. The anatomy of the shoot apex with a single discrete tunica layer presents a high type of organization for angiosperms. The occurrence of numerous morphological adaptations to the dry habitat supports the concept of the specialized nature of the genus. An objective consideration of the cumulative evidence and its analysis should convince one that Garryaceae can no longer be considered primitive.

Many systematists think that the Angiospermae must have had a polyphyletic origin. The evidence is found in the great diversity of families and their

long geological history which goes nearly as far back as the angiosperms have been reported. In addition, the difficulty of relating certain families, such as the amentiferous ones, to others causes many to rely too implicitly on the polyphyletic hypothesis and not to exhaust all possibilities of closer relationships.

It may be true that many of the modern angiospermous families, or the trends leading to their differentiation, arose more or less simultaneously (Stebbins, 1950) relatively early in angiospermous evolution (Arnold, 1947; Axelrod, 1952; Darrah, 1939; Stebbins, 1950). Perhaps, the basic trends which arose simultaneously were manifested in several generalized taxa which we would now term ordinal or supraordinal, such as the suggested pro-umbelliflorean ancestor. If a polyphyletic conception refers to this thesis, something different is referred to than a strictly polyphyletic origin of the flowering plants. Such a theory refers to the origin of the majority of modern families, or trends leading to them, not to the origin of angiosperms. We are still confronted with the question of which taxon gave rise to the first angiosperm or angiosperms. Stebbins (1950) thinks of an original stock of Jurassic age, relatively similar, with ranalian characteristics from which rapid differentiation of familial taxa occurred. What preceded the primitive complex? Was it a group with strobiloid, hermaphroditic, hypogynous, entomophilous, dichlamydeous flowers or was it a conifer-ephedroid type with unisexual, achlamydeous, dioecious, epigynous, and anemophilous flowers?

Evidence of primitive features in the Amentiferae would support an amentaceous origin for the pre-ranalian complex, under either polyphyletic or monophyletic hypotheses. A preponderance of specialized features in the Amentiferae, especially of a reduced nature, would preclude the idea of a pre-ranalian amentaceous stock which gave rise to the ranalian complex, might at least question ideas of a separate origin of amentiferous types, and would substantiate claims of the primitive, direct origin of the ranalian complex. During recent decades there have been thorough studies of the Amentiferae, especially of their secondary xylems and floral anatomies. The work has been productive and important because the "tools" of study are more reliable than those of gross morphology and the evidence relatively unbiased, clear, and convincing. As described previously, studies of this sort have revealed decisive and vindciating evidence of a moderate or high degree of specialization among amentiferous taxa. The evidence has been so defensible in most cases as to preclude any further serious consideration of the amentiferous families as representatives of primitive angiosperms or vestiges of transitional forms from which arose dichlamydeous, polypetalous, hermaphroditic-entomophilous taxa. Recently, Bailey (1949, 1954) has pointed out that the omission of scalariform tracheids in the normal sequence of tracheid evolution in the coniferous and ephedroid gymnosperms, and the widely accepted evidence that the angiospermous vessel element has arisen from the scalariform tracheid (Bailey, 1944a, 1944b, 1949, 1953, 1954; Bailey & Nast, 1945; Bailey & Tupper, 1918; Cheadle, 1943; Frost, 1930; Thompson, 1918; Thompson & Bailey, 1916), have greatly decreased probabilities of angiospermous derivation from coniferous or ephedroid ancestors. Parkin (1952) has also described obvious evidence contributing to the same conclusion. In no angiosperm with perfect flowers has the relative positions of the floral organs been altered. If hermaphroditic flowers arose according to the pseudanthien theory, there is no reason why some flowers should not have stamens above carpels. This has never happened. To hold to Neo-Englerian views and to the pseudanthien theory is to ignore exceptionally sound evidence in favour of largely exomorphic and often admittedly deceptive evidence.

The Garryaceae are associated with the Amentiferae, and considered primitive by most adherents to Englerian and Neo-Englerian systems. The family offers, under careful scrutiny, however, many indications of specialization sufficient to repudiate retention of the family in a primitive position. Once the evidence

is clear, one is compelled to find a more logical position for the family. The task is a little less formidable if one accepts a polyphyletic view for the origin of the Angiospermae. In considering the several very constant characters found throughout, or in the majority of, angiosperms, such as the 8-nucleate monosporic embryo-sac, porogamy, " double fertilization", non-motile sperms (Maheshwari, 1948, 1950), the monophyletically derived vessel element of the dicotyledons (Bailey, 1949), one feels restrained from utilizing the polyphyletic theory, at least in an extreme form. One realizes that an acceptance of an extreme polyphyletic theory for the origin of the flowering plants is just as unrealistic as holding to a strictly monophyletic theory.

The Amentiferae are not a natural group. The differences among the families are too great and many similarities only apparent. Even if the polyphyletic concept for the origin of the angiosperms is correct, at least some of the amentaceous families present convincing evidence of affinities with other families. The Garryaceae, for example, once removed from the Amentiferae and a primitive position for the many reasons described, should not be derived independently from ranalian stock when evidence supports close genetic relation to the Cornaceae.

Summary

1. The secondary xylem of *Garrya*, only genus of the Garryaceae, is described. In the discussion the xylic characters are listed and classified with regard to their phyletic level.

2. The secondary phloem anatomy and nodal structure are also described.

3. *Garrya* has been linked variously with Cornaceae, Amentiferae, Celastrales, Hamamelidaceae, and Caprifoliaceae. Utilizing the original descriptive material of the secondary xylem, secondary phloem, and node, coupled with available information obtained from other botanical disciplines, the Garryaceae are compared with these principal taxa.

4. The cumulative, comparative evidence strongly favours a relationship of Cornaceae and Garryaceae over all other suggested alliances. The greatest number of similarities occur in these two families. Where similarities are lacking, clear, logical sequences of specialization from the Cornaceae to Garryaceae are often evident. It is thought that the Garryaceae have differentiated by reduction from a pro-umbelliflorean ancestral complex, cornaceous in nature.

5. The phyletic level of Garryaceae and its bearing on the problem of the origin of the Angiospermae is considered. The family has often been placed in the Amentiferae by systematists who consider amentaceous taxa primitive or transitional angiosperms. *Garrya* offers, however, sufficient indications of specialization to repudiate retention of the family in a primitive position. Moderate specialization along lines of reduction has lead to its amentaceous nature. The secondary xylem, for example, has very few primitive characters, many moderately advanced ones, and very few highly specialized ones. Available evidence from phloem anatomy, floral anatomy, pollen morphology, developmental anatomy, ecological structure, and, to a lesser extent, nodal structure support the thesis of a moderately advanced phyletic level for the family.

6. As similar overwhelming evidence of specialization in many other amentiferous families has been discovered in recent years, it is not unreasonable to consider the pseudanthien theory for the origin of the flower and the Englerian system of classification untenable or at least to expect an objective reconsideration of these concepts.

It is a pleasure to acknowledge the several persons who obtained wood samples for this research, the American Academy of Arts and Sciences and the University of California, Santa Barbara College, for grants-in-aid of research, and the Santa Barbara Botanic Garden for research facilities. We are indebted to Mrs. Jean M. Moseley, Dr. C. H. Muller (University of California, Santa Barbara College), Dr. G. N. Jones (University of Illinois), and Dr. O. Tippo (Yale University) for critical readings of the manuscript.

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KALLOSTACHYS SCOTTII: A NEW GENUS OF SPHENOPSID CONES FROM THE CARBONIFEROUS

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In the course of a comprehensive survey of Carboniferous spores in structurally preserved fructifications a cone of unusual structure and morphology has recently been observed. The cone is represented by three sets of slides, two transverse and one longitudinal. The slides had been prepared some years ago in connection with Mr. W. C. Darrah's studies of American Carboniferous petrifactions and had been deposited among a larger number of undescribed specimens in the Botanical Museum of Harvard University. A total of fifty-nine slides were available for study, in addition to part of one cone preserved in a coal ball. Two cones had originally been sectioned, as deduced from the two sets of transverse sections. Preservation is sufficiently good to make possible restoration of the basic morphology of the cone and the significant details of its anatomical structure. The slides are numbered 55575-55634 and are deposited in the Palaeobotanical Collections of Harvard University.

The diameter of the cone, including bracts, as measured in transverse section, is approximately 20 mm; the diameter of

the cone axis from the same section measures approximately 7 mm. In longitudinal section, however, the width of the axis is about 4 mm. The discrepancy between these two measurements is due to compression, thus giving one axis of the transverse section a greater diameter than the other. Because of its incompleteness, the exact length of the specimen cannot be determined, but is at least 3 cm and probably more.

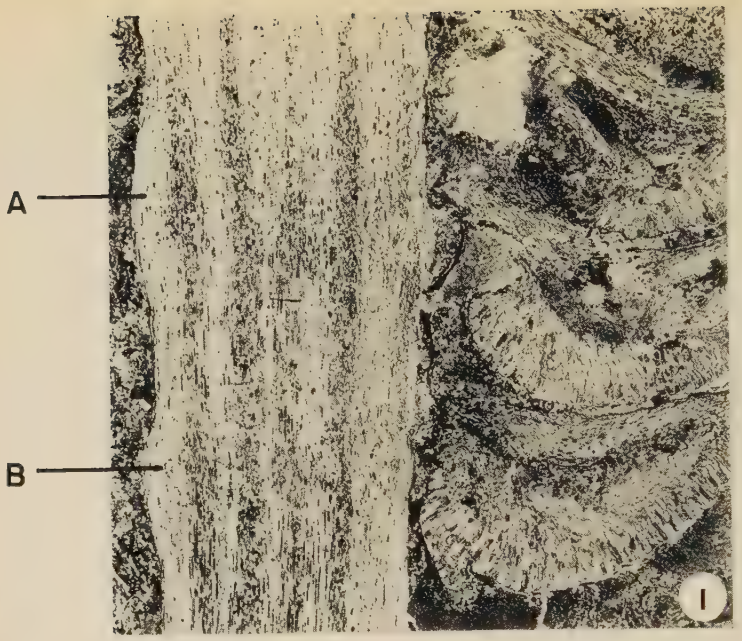
The petrifaction under consideration is from a coal ball collected in the Shuler Mine, and is stratigraphically assignable to the Des Moines Series of the Middle (?) Pennsylvanian of central Iowa (Moore *et al.*, 1944). In terms of European chronology, the Des Moines Series is correlated with the upper Westphalian C or lower Westphalian D of western Europe. The Shuler Mine, now inoperative, is in Walnut Township, Dallas County, Iowa. From the same geographic region and from numerous coal seams, presumably representing stratigraphic equivalents abundant petrifactions rich in plant remains have been collected in the past; in fact the major part of our knowledge of structurally preserved Carboniferous plants from the United States has been derived from calcareous petrifactions occurring in the mid-continent coalfields of Iowa, Illinois, Kansas and neighbouring states.

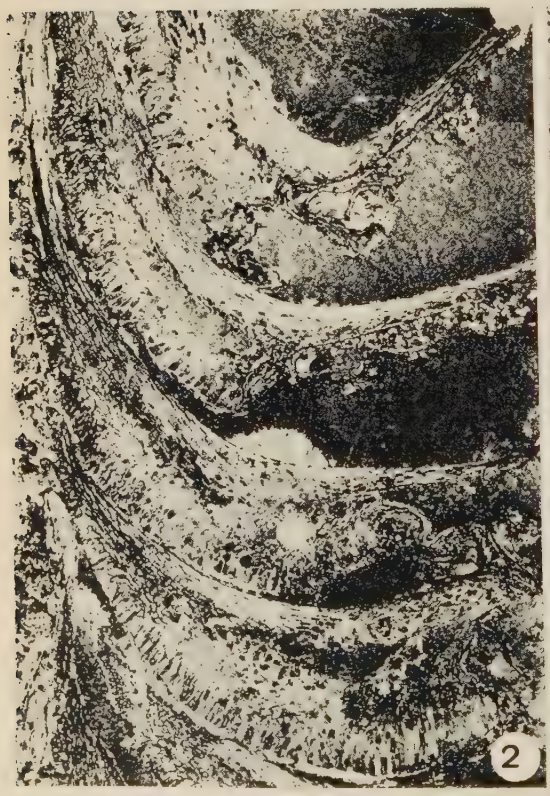
Although it was possible to work out in considerable detail the morphology and anatomy of the fossil cone, there is no evidence from the sections, nor from the remaining portion of the cone in the coal ball, which suggests the identity of the vegetative plant to which the fructification was attached. The genus is placed in the subphylum Sphenopsida on the basis of certain gross structural similarities which it possesses in common with the reproductive organs of this group of articulate plants. The cone is not comparable to any known member of the group; however, for in many significant details, it differs from all Sphenopsid cones both living and fossil.

General Morphology

The cone consists of sporophylls arranged in whorls on an axis characterized by distinct nodal-internodal organization (Fig. 1). Twelve sporophylls are present at each node; the sporophylls comprising one whorl are basally fused for an undeterminable distance. The whorls are approximately 2 mm apart and the distally extended tips of the sporophylls from one whorl overlap those of the next whorl above for a considerable distance. Sterile bracts are absent. The sporangia, of which there are two per sporophyll, occur somewhat embedded in the *abaxial* surface of the sporophyll, although they are not completely enclosed by sporophyllar tissue (Fig. 2). The abaxial insertion of the sporangia in conjunction with whorled organization of the cone distinguishes this genus from any known Carboniferous fructification. It will be noted that the sporangium-bearing organ is referred to as a sporophyll. In many of the Sphenopsida, the term sporangiophore is widely used to designate the fertile appendage. Since no sterile appendages are present in this genus and since the sporangium-bearing organs show no relation to the sporangiophoric structure and are of a truly foliar nature, it seems more appropriate to designate them " sporophylls ". The sporangia are radially elongate structures which, when intact, contain an abundance of spores. The spores are of a type found in a number of fruiting structures definitely assignable to the Sphenopsida, such as *Discinites* (Arnold, 1944), *Calamostachys* (Schimper, 1869), *Macrostachya* (Schimper, 1869) and *Paleostachya* (Weiss, 1876). The spores may be included in the genus *Calamospora* as defined by Schopf, Wilson & Bentall (1944). Inasmuch as the cone contained only one type of spore throughout, it may reasonably be assumed that the plant was homosporous. The possibility of heterospory, however, cannot be completely eliminated from consideration in view of the fact that the material at hand does not represent a complete specimen.

Despite the fundamental Sphenopsid organization of the fertile appendages, the axis of the cone is more lycopsid-like in organization. As in the vegetative axis of *Sigillaria*, the cone is characterized by a hollow pith, exarch primary xylem, of which there is an amount proportional to





FIGS. 1-3.

FIG. 4 — T.s. axis showing 12 exarch bundles of eustele with hollow pith and radial rows of secondary wood. × 12.

that found in stems of *Sigillaria*, and also by the presence of a small amount of secondary wood. As is ordinarily the case with Carboniferous plants, the phloem is not preserved. In longitudinal section throughout the primary and secondary wood of the cone axis, small spore-like bodies were observed, some of which possess hyphal extensions. These structures indicate that the axis was probably invaded by a fungus prior to deposition and burial. Whether this fungus attacked the living tissue parasitically or entered during the early stages of degradation cannot be determined.

THE AXIS (Figs. 4, 7) — The axis of this cone is characterized by a large hollow pith, a situation similar to that found in *Calamites* and most species of *Equisetum*, the cavity resulting from breakdown of tissue during growth and differentiation. The primary xylem is exarch in organization and consists of a considerable number of protoxylem elements which occasionally are centered around a protoxylem or carinal canal. However, the number of protoxylem elements is greater than the number commonly found in the protoxylem or carinal canals of living representatives of the Sphenopsids. The extent of protoxylem development, however, is comparable to that found in *Calamites*. Since the metaxylem is quite extensive, the axis differs markedly from either

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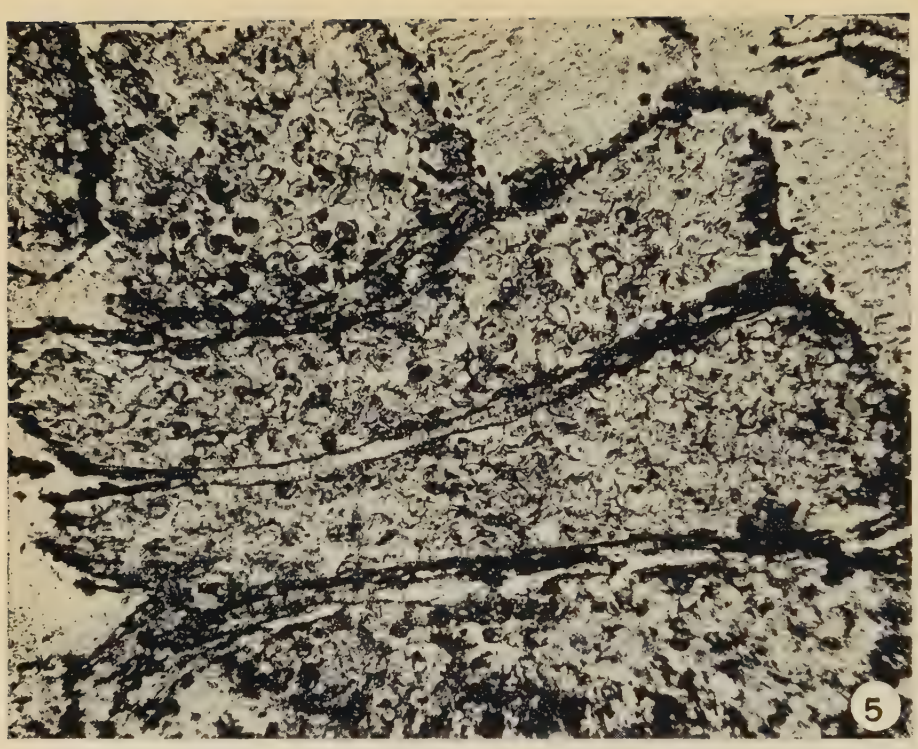
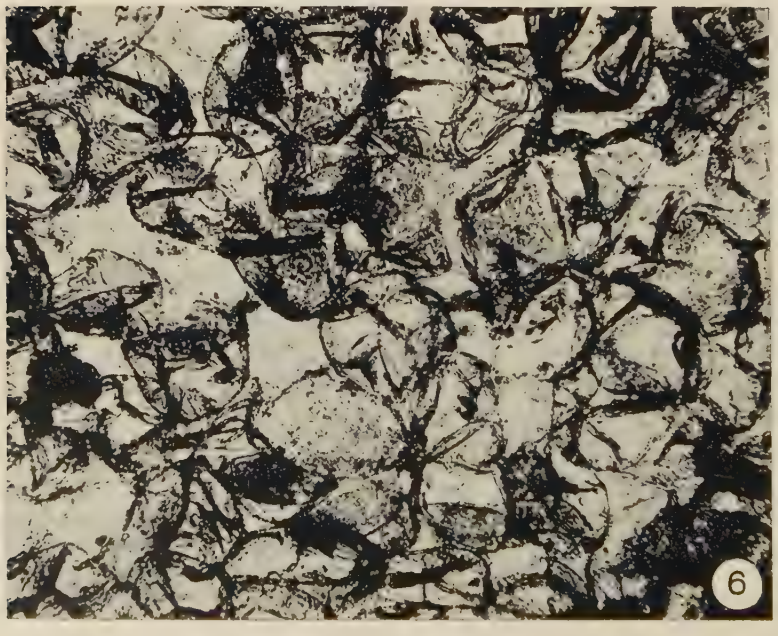
FIGS. 1-3 — Fig. 1. L.s. portion of cone showing relation of sporophylls to axis and whorled arrangement of traces in axis. Arrows A and B point to 2 successive nodes. × 10. Fig. 2. L.s. showing structure of sporophylls and abaxial position of sporangia. × 10. Fig. 3. T.l.s single nodal trace in the axis. × 300.





FIGS. 5, 6.

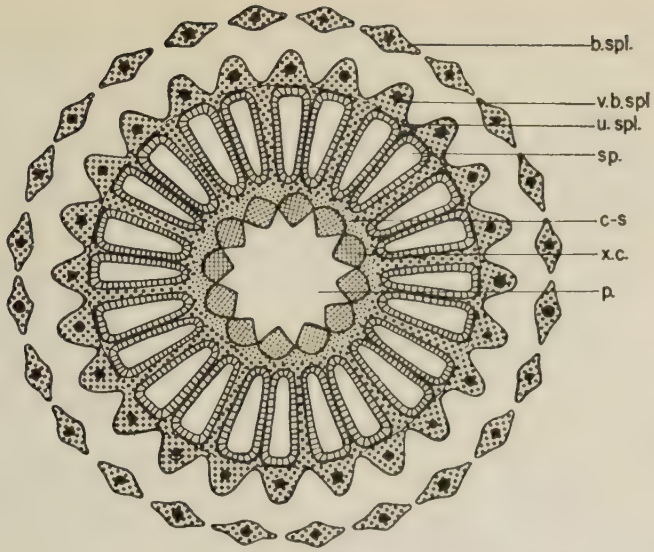


FIG. 7 — Diagramatic t.s. cone (*b. spl*, bifurcated sporophylls; *v.b. spl*, vascular bundle of sporophyll; *u. spl*, sporophylls fused at base; *sp*, sporangia; *c-s*, cortical and sporophyll tissue; *x.c*, xylem cylinder; *p*, pith). × 3.

Equisetum or *Calamites*. In this respect, the axis of the cone is more comparable to Lycopsid than to Sphenopsid organization. Secondary xylem is not as extensive as the primary xylem. The secondary xylem, in transverse section, appears to form caps over the primary xylem thus giving the vascular cylinder a lobate appearance. Medullary rays extend through the secondary wood and terminate in poorly defined openings in the cortical tissue comparable to vallecular canals. The parenchymatous walls of the ray cells are not always preserved, but where they are present, the rays appear to range from approximately 3-8 cells in width, at their widest dimension.

The vascular cylinder of the cone, hence, is an exarch eustele featured by protoxylem arranged around small protoxylem canals, and by a cortex with "vallecular canals". Surrounding the primary wood is a limited but significant amount of secondary wood.

In longitudinal section, scalariform thickenings were observed on the tracheid walls of the xylem in the cone axis; it was not found possible to distinguish between metaxylem and first formed secondary xylem.

THE SPOROPHYLL — From transverse sections, it can be demonstrated that the sporophylls are fused at their base and are coalescent for probably one-half of their extent (Fig. 8). The upper portion of the sporophyll is free and tapers to a bifurcated narrow tip. Each sporophyll bears two sporangia radially elongated and tangentially appressed to each other (Fig. 5). Vascular strands are clearly distinguishable only in the free tips of the sporophylls. Unfortunately, it was not

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FIGS. 5, 6 — Fig. 5. T.s. narrow and radially elongate sporangia. The sporangial walls are one-cell thick. Sporangia are filled with spores assignable to the genus *Calamospora*. × 35. Fig. 6. Cluster of spores from *Kallostachys scottii*. Note the folds in exosporial walls due to collapse of the spores, and the triradiate marks. × 300.

possible to determine vascularization of the sporangia. Each whorl, comprising the basally fused sporophylls, consists of a thin sheet of tissue thickening conspicuously beyond the region of insertion of the abaxially developed sporangia. Successive sporophyll whorls are inserted on the axis more or less at right angles and are extended vertically with an upward, abrupt curve. The long, slender, free tips overlap the sporophylls of the superseding whorl for a considerable distance. Epidermal and subepidermal cells of the adaxial portion of the sporophyll are conspicuously elongated in the direction of curvature of the sporophyllar whorl. The lower, morphologically dorsal, surface is characterized by epidermal cells elongated at right angles to the axis of curvature (Fig. 2). Immediately adjacent to the tangentially paired sporangia, the sporophyll possesses a pronounced keel of parenchymatous tissue extending the full width of the sporophyll. The "keel" tissue does not extend radially beneath nor encroach upon the sporangial cavity so as to enclose or embed it. At the bottom of the "keel" and extending upward to the tip of the sporophyll on the abaxial side the layer of palisade-like cells becomes progressively radially shorter toward the tip (Fig. 8). Between this layer of cells and the uppermost layer, the sporophyll tissue, structurally preserved, consists essentially of parenchyma. The central parenchymatous tissues are not preserved as well as the peripheral cell layers in any of the sporophylls, a structural condition probably resulting from differential degradation of the cell walls in the several tissue layers at the time of the peat stage and before mineralization.

The Sporangia (Fig. 5) — Although sporangial walls have been found attached to the abaxial surfaces of the sporophylls, as seen in longitudinal sections, the sporangia themselves are preserved intact in only a few of the transverse sections available. In several of the transverse sections, masses of spores may be observed which are appressed between the axis and the sporophylls, with all traces of continuous sporangial walls absent. It may be inferred from this that the sporangia were closely appressed and that a relatively small amount of compaction would cause crushing of the sporangial walls with resultant release of spores. The sporangia are radially elongate structures approximately 3 mm long, tangentially widening at their distal end. The sporangial wall consists of a single layer of cells, the constituent cell walls of which are moderately thick.

Spores (Fig. 6) — Spores in occasional intact sporangia are very numerous. They are virtually devoid of definable ornamentation and possess a triradiate attachment scar. They are radially symmetrical, spherical, but often characterized by sharp folds of the exosporial wall. The spore coat is quite thin. In many respects, the spore characteristics conform closely to *Calamospora*, a form genus proposed by Schopf, Wilson & Bentall (1944). Nemejc (1937) and Arnold (1944) have illustrated spores isolated from compressions assigned to the genus *Discinites* of the Noeggerathiales, which resemble the spores in the new genus here described. Structural features of the spores of this cone, though not of critical value, support the inclusion of this reproductive structure in the Sphenopsida.

Kallostachys scottii[1] Brush and Barghoorn, gen. et. sp. nov.— A cone, the diameter of which is approximately 20 mm, and length unknown owing to incompleteness of the specimen. The axis is 6-7 mm in diameter. The vascular cylinder consists of an exarch eustele with hollow pith. The primary xylem strands are flanked by a considerable amount of secondary wood. The protoxylem consists of a few tracheary elements arranged around a protoxylem canal. All tracheids, both of metaxylem and secondary wood exhibit scalariform pitting.

Broad interfasicular segments dissect the vascular stele from the pith to the cortex. Canals which are analogous to the vallecular canals of *Equisetum* are present in the cortex. The axis is characterized

1. The generic name is from the Greek "kallos" meaning "beautiful" and from "stachys", "cone". The specific name is in recognition of D. H. Scott who first designated the articulate plants as Sphenopsida.

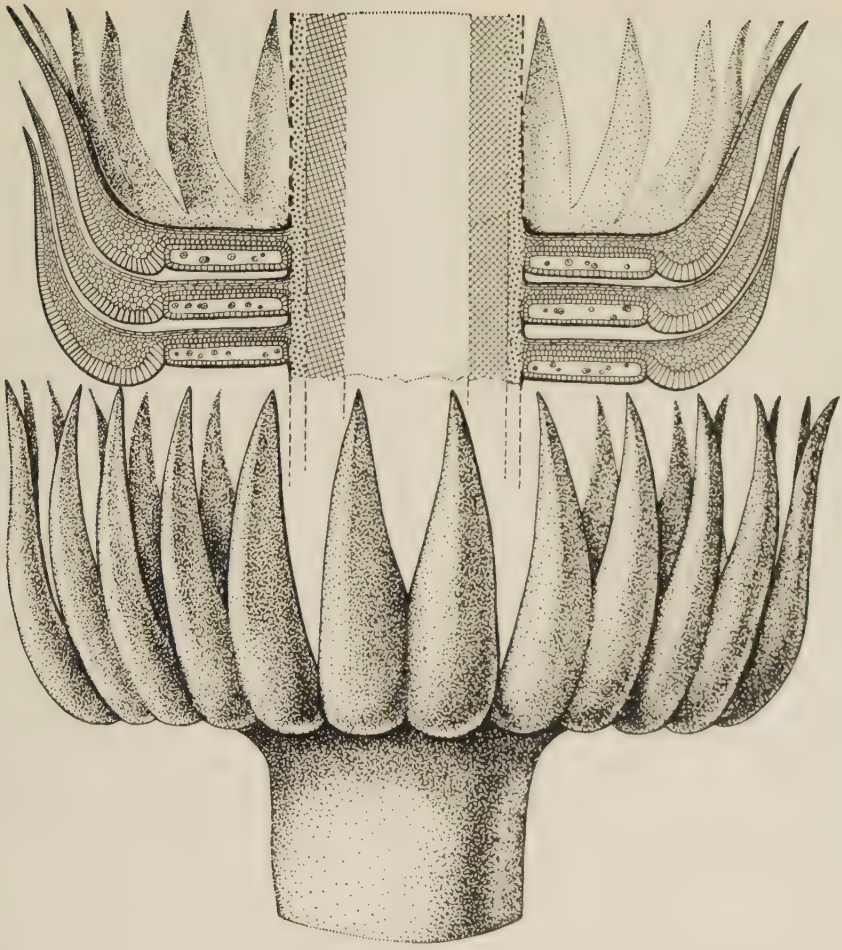

FIG. 8 — A reconstruction of a portion of *Kallostachys scottii*. Note sporangia embedded in abaxial surface of sporophylls. × 4.

by distinct nodal-internodal organization in repeated whorls. There are 12 sporophylls per whorl, basally united, but dichotomizing and free at the tips. Sterile bracts are absent. Two sporangia are borne on the abaxial, morphologically dorsal side of each sporophyll. The sporophylls are characterized by a large keel at the base, and their free tips imbricately overlap those of the superseding whorl for a considerable distance. The sporangia are radially elongated sacs about 3 mm long and 0·5-1 mm wide. There are 24 sporangia per whorl. The cone is apparently homosporous with spores averaging 70-80 μ in diameter. Spores are thin-walled, possess a trilete attachment scar, and are featured by folds in the exosporial wall. On the basis of systems of spore classifications, the spores would be assignable to the genus *Calamospora*.

Locality and Horizon — Upper Carboniferous, Des Moines Series, equivalent to the upper Westphalian C or lower Westphalian D of Europe, Shuler Mine, Walnut Township, Dallas County, Iowa.

Collected by — Frederick Oliver Thompson.

Material — One petrified specimen from a coal ball and slides prepared from two specimens.

Genotype — Slides made from peels in the Palaeobotanical Collections of Harvard University, No. 55575 to 55634.

Discussion

As suggested in the previous description of the proposed new genus, the affinities of *Kallostachys scottii* to the Sphenopsida are based more on fundamental morphological relationships to this group of lower vascular plants than on specific similarities to any known member of the group. As defined by Scott (1909) and subsequently amended by other authors, the Sphenopsida comprise a group of vascular plants characterized by pronounced nodal-internodal differentiation, or articulate organization throughout the plant body; by ridged and grooved external structure of the aerial stems, and sporangia borne on stalked appendages inserted in whorls on terminal strobili. Sterile appendages on the strobili may or may not be present, but if present, are likewise borne in whorls inserted alternately with the whorls of fertile appendages. The fertile structures have been designated sporangiophores.

The cone *Kallostachys scottii* fits the morphological concept of the Sphenopsida with respect to its articulate organization of nodes and internodes. It cannot be determined whether the external morphology of the cone axis was featured by ridges and grooves. The sporangium-bearing organ of *Kallostachys scottii*, however, may scarcely be regarded as a sporangiophoric structure, but more aptly as a sporophyll. In basic organization, it is very similar to the sporophylls of the Lycopsida, except for the position of insertion of the sporangial sacs on the lower rather than the upper surface. The exact morphological nature of the sporangiophore in Sphenopsid plants is controversial. Bower (1908) maintains that there is no definite relation between bracts and sporangiophores. Benson (1908) regarded the sporangiophore as a fundamental unit which may or may not be united with the leaf. Scott (1920) regarded the sporangiophore as a leaf or a lobe of a leaf. Goebel (1930) describes sporangiophores as fertile leaves.

In view of the varied interpretations of the morphology of the Sphenopsid cone, the absence of sterile bracts in *Kallostachys scottii* may be interpreted in a number of ways: (1) the cone may be a derivative of *Pothocites* if one interprets this genus as having been primitively bractless. If such is the case, the sporangia have proceeded from a primitive terminal psilophytalean condition to the adaxial position of *Pothocites* and thence to the abaxial insertion in *Kallostachys scottii*. (2) This genus may show a further development of the *Calamostachys* line by loss of sterile bracts. The sterile bracts of *Calamostachys* and *Paleostachya* are believed by some to be lobes of dorsally divided sporophylls. Evidence for this is derived from the structure of the genus *Cingularia*. In *C. cantrilli* sterile bracts are absent, whereas in *C. typica* the lower division of this cleft sporophyll has become sterile. *Equisetum* itself could also fit into this line of phylogeny. (3) The sterile and fertile bracts, such as in *Calamostachys*, etc., may represent a primitive line and by fusion of both, the presence of fertile bracts with no sterile appendages has arisen as, for example, in our new genus *Kallostachys scottii*. Whatever the situation may be with respect to sterile and fertile appendages, it is futile to attempt an interpretation of *Kallostachys scottii* based on the absence of sterile bracts, if the characteristic position of the sporangia is to be regarded as of major significance within the Sphenopsida.

The detached cones of the Sphenophyllales, such as *Bowmanites*, bear little more than minor resemblance to *Kallostachys*. *Bowmanites bifurcatus* described by Andrews & Mamay (1951) is similar in that the upper portion of the sporophyll is bifurcated. Although, in *Bowmanites*, the cone axis is articulate with short internodes, sterile bracts are usually present and commonly united with the sporangiophores. Moreover, each sporangiophore or sporangiophoric branch bears a single oval sporangium terminally on its tip. *Cheirostrobus* (Scott, 1897), *Peltastrobus* (Baxter, 1950), *Litostrobus* (Mamay, 1954) are also Sphenophyllalean

cones, to which *Kallostachys scottii* bears little similarity beyond its articulate organization.

All fructifications of the Calamitales possess a central articulated axis, and sporangiophores borne in whorls. In *Calamostachys*, sterile bracts are present and are joined for some distance but the tips are free. The sporangiophore whorls occur equidistant between the sterile whorls. *Paleostachya* differs from *Calamostachys* in that the sporangiophores are borne in the axils of basally fused sterile bracts. *Macrostachya* similarly is characterized by the presence of sterile bracts. In all three of these genera the sporangia are borne reflexed on the sporangiophore, and are extended towards the cone axis. *Cingularia*, which has already been discussed, has highly modified sporangiophores. It is, therefore, clear that *Kallostachys scottii*, although related to the Sphenopsida, in general, bears little resemblance to the fructifications of either the Sphenophyllales or Calamitales, and it is, therefore, not possible to place it in either of these groups. With respect to the Noeggerathiales, whose position in the Sphenopsida itself is not well founded, the following characters exclude any relation to *Kallostachys*: non-articulated axes, adaxial (morphologically ventral) position of the sporangia, and in the case of *Tingia* and *Sphenostrobus*, tetrarchous protosteles.

With respect to the cone axis, *Kallostachys* resembles *Calamostachys* in possessing a central pith and pith cavity.

The genus *Kallostachys* is, therefore, placed in the Sphenopsida in conformity with the original characterization of the group by Scott. These fundamental features are simply articulation of the stem and whorled organization of appendages (Scott, 1909). The structure of the spores is of the type found in *Calamostachys*, *Discinites*, and certain other fossil Sphenopsida, viz. smooth, unsculptured exine, frequently sharply folded, and possessing a triradiate scar.

Summary

A petrified cone from the Pennsylvanian Des Moines Series of Iowa is described as a new genus and placed in the subphylum Sphenopsida. The new genus, *Kallostachys*, is characterized by an articulate axis, with an exarch eustele and fistular pith. The sporangia are embedded in the abaxial surface of the sporophylls. Spores found within intact sporangia are assignable to the genus *Calamospora*, a form genus based on isolated spores now known to be referable to several distantly related genera. The genus is represented by one species, *Kallostachys scottii*, and by two specimens. Although the specimens are sufficiently preserved for a detailed anatomical study, they were not found attached to vegetative organs. The genus is placed in the Sphenopsida on the basis of fundamental structural organization, but it cannot be assigned to any recognized order of this subphylum. The genus is significant in enlarging our concept of the morphological range achieved in Palaeozoic representatives of the subphylum Sphenopsida, particularly with reference to the position of attachment of the sporangia and its relation to the controversial concept of the sporangiophore.

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THE MORPHOGENETIC EFFECTS OF VARIOUS PHYSICAL FACTORS ON THE GEMMAE OF *LUNULARIA*[1]

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In the course of recent investigations we found that the gemmae of *Lunularia cruciata* are subject to many morphological alterations in response to treatment with diverse chemicals. From this work the question naturally arose as to whether these gemmae could be modified by physical factors in any comparable fashion. Nagai (1919) found that plasmolysis was a strong morphogenetic factor on *Marchantia* gemmae. Dickson (1932) did further work on plasmolysis of *Marchantia* and in addition studied the effects of X-rays, ultraviolet rays, and of hydrogen-ion concentration. Fitting (1942) and Halbsguth (1936, 1953) have studied the effects of position, contact, light, etc., on the development of dorsiventrality in thalli produced from gemmae so treated. But the morphogenetic influences of numerous physical factors appeared to remain unexplored, and as a result, we have investigated those which could be studied with the facilities available.

Methods

The gemmae were removed from the gemmae cups, subjected to treatment as described later under the different headings, and then grown on filter paper spread on quartz sand moistened with the mineral constituents of White's (1943) solution. Wherever we speak of White's solution, it is to be understood that we mean only the mineral substances of that solution. The petri dishes were kept in a greenhouse built on the north wall of the laboratory building where they received practically no direct sunlight.

Camera lucida drawings have been made using a Bausch & Lomb wide field stereoscopic microscope.

1. Paper No. 1033 from the Department of Botany, University of Michigan. This work was aided by a grant of funds to C. D. LaRue from the Rackham Research Fund.
2. Fulbright Fellow from the University of Delhi, India, 1953-54.

Observations and Results

THE EFFECTS OF VARIED HYDROGEN-ION CONCENTRATIONS — Gemmae were grown in petri dishes on filter papers soaked with buffered White's solutions. The solutions used, ranged from *p*H 2·5 to *p*H 13 in half unit steps. Growth at *p*H 11·5 was much inhibited and at *p*H 12, 12·5 and 13, all the gemmae died within a few days. At *p*H 2·5 also, the gemmae did not survive. No noticeable variation could be seen between gemmae grown at any one *p*H and any of the others within the range of tolerance. This shows a rather remarkable range.

THE EFFECTS OF CENTRIFUGING GEMMAE AT HIGH SPEEDS — Gemmae were centrifuged at 28,500 times G for 5, 10, 20, 40, 60, 80 and 100 minute periods and then grown in the usual way. No effect whatever could be detected from this treatment.

EFFECT OF SUBJECTING GEMMAE TO VACUUM — Gemmae were spread out on moist filter paper and kept in a chamber, and air exhausted for varying periods. Later they were taken out and grown on sand culture, irrigated with White's solution.

Vacuum Treatment for 12 *Hours*—Growth of gemmae from apical growing points was retarded. However, normal thalli were formed, though somewhat thickened and green. No rhizoids were formed on the upper surface.

Vacuum Treatment for 24 *Hours*—Many gemmae were dead, as indicated by the loss of green colour. However, in some the apical growing points were alive and produced small, succulent buds, later developing into normal thalli. Growth was considerably slowed down.

Vacuum Treatment for 36 *Hours*—Mostly all gemmae were dead, but occasionally a few were green and appeared to show no effect. These were apparently highly resistant to vacuum conditions.

MUTILATION

Effect of Slicing off Growing Points after Gemmae had Grown for 10 *Days*—Rhizoids arose from the upper surfaces. On each thallus a bud or two arose from the upper surface, usually near the cut ends.

Slicing into Pieces — Gemmae which had been grown for one week were sliced crosswise into 4 pieces. Further growth resulted in pieces of unequal size from each original gemma. A growing point developed into a thallus from the uncut end of each piece (Fig. 1 A, B). From gemmae which had been sliced lengthwise into 3 pieces, thalli developed with growing points at each end. In some pieces, extra growing points and split tips developed (Fig. 1 C, D). When gemmae were sliced into very small pieces each piece produced a cylindrical, upright lobe which was button-like in appearance but soon widened out into a thallus (Fig. 1 E). The results of slicing gemmae crosswise are shown in Fig. 2 A, B.

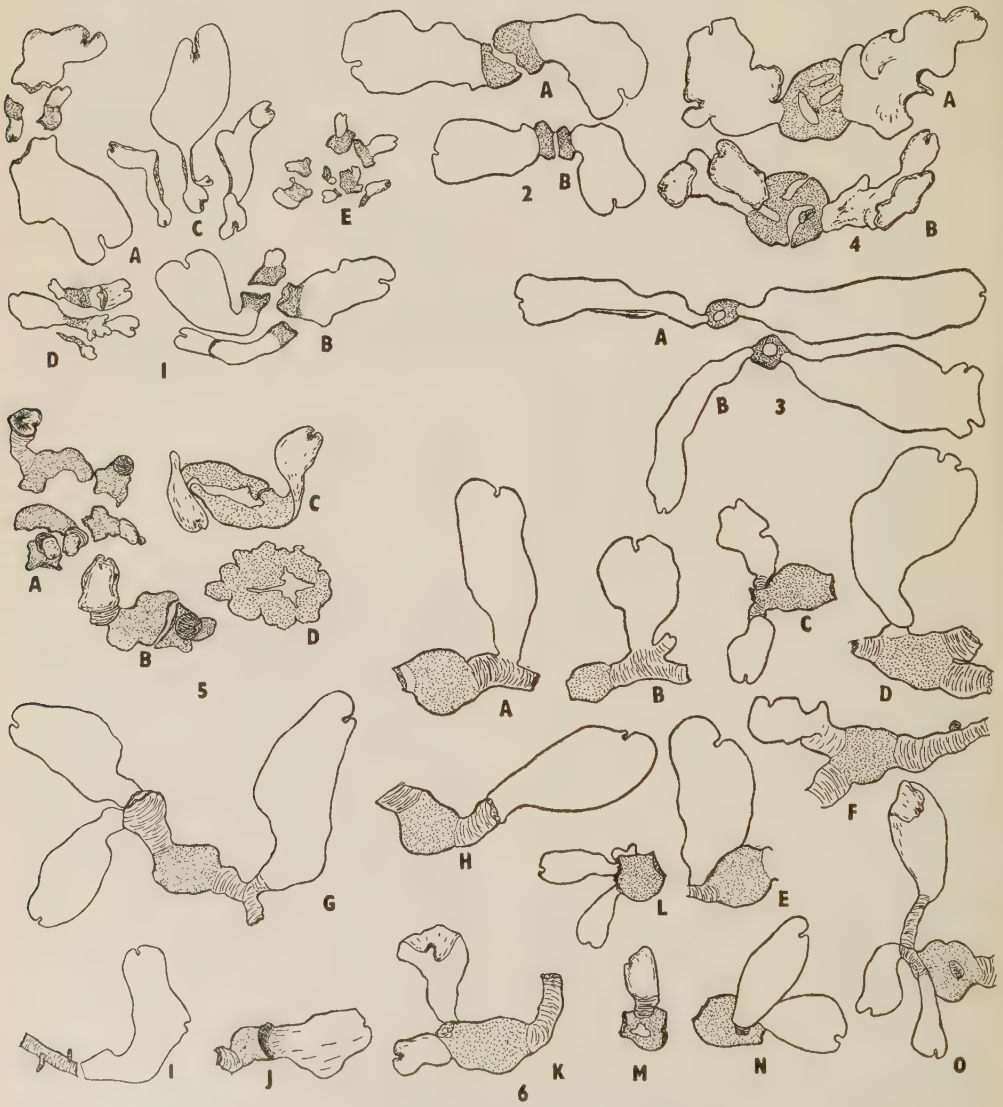
Puncturing of Gemmae — The gemmae were punctured with a fine needle. It was thought that buds might develop on the periphery of the puncture, but they did not. The puncture remained open and thalli grew out from the ends in normal fashion (Fig. 3 A, B). Compare these with Fig. 4.

Slits in Gemmae — Where the slits did not have a special isolating effect, the wounding led only to irregular growth of the thalli produced from the growing points (Fig. 4 A). Where the cuts caused isolation of the outer ends of sectors of gemmae leaving the inner parts intact, each isolated outer end formed an irregular upright bud, which later unfolded into a regular thallus (Fig. 4 B).

Slicing Followed by Chemical Treatments — Gemmae were sliced into pieces, then treated with colchicine, 1×10^{-2} M, and grown for six weeks. Each piece developed into an upright, cylindrical lobe. These lobes soon began to expand at the top into irregular forms similar to those we have previously described (1955) as " cups " and " feet " (Fig. 5 A, B). Gemmae sliced lengthwise and treated with IAA, 1×10^{-3} M, and grown for three weeks to one month gave somewhat similar results (Fig. 5 C), but the outgrowths were more thallus-like from the beginning, standing upright and at right angles to the lengthwise plane of the gemmae, thus resembling " feet " with narrow heels. Slits were made in gemmae

which were then treated with NAA, 1×10^{-5} M. This resulted in growth of the bodies of the gemmae in all directions, but all growing points were inhibited (Fig. 5 D).

Amputation of "Feet" — In a previous paper (1955) we have described the development of upright, cylindrical lobes which terminated in expanded portions which reminded one strongly of human feet. We first saw these developed as a result of growing gemmae in diffuse light on a klinostat. Later we saw these structures formed as a result of various different treatments. The removal of the expanded ends of the upright lobes



FIGS. 1-6 — Effects of mutilation on growth of gemmae. Fig. 1 *A-E*. Sliced into pieces; *A, B* sliced at right angles to each other and *C, D* sliced lengthwise, and *E* sliced into pieces. × 14. Fig. 2 *A, B*. Halved crosswise. × 7. Fig. 3 *A, B*. Puncturing of gemmae. × 7. Fig. 4 *A, B*. Slits in gemmae. × 14. Fig. 5 *A, B*. Slicing followed by chemical treatments. × 14. Fig. 6 *A-O*. Amputation of "feet". × 14.

(the " feet ") resulted in the regeneration of new buds or thalli from the remaining parts. If the " foot " was excised from the outer end of the cylindrical lobe (" leg "), regeneration from the lobe itself occurred (Fig. 6 A-H). If the lobes were cut off near the gemmae, new thalli grew out from the cut ends of the gemmae (Fig. 6 J-L). Regeneration of two buds on one stalk is shown in Fig. 6 I. Fig. 6 J shows a regenerated thallus which has split immediately after its formation. The nature of this splitting, actually a new type of branching, has been discussed in our previous papers (1954, 1955). In Fig. 6 M-O, upright lobes on bodies of the gemmae are shown. These developed after amputation of both " feet " and " legs ".

Removal of Growing Points — Gemmae were grown for 5 days, then growing points were removed by cutting across the ends. This treatment resulted in the formation of numerous buds on the cut ends of the gemmae (Fig. 7 A-C), the buds developing into thalli later.

When gemmae were grown for 10 days before excision of the growing points, fewer buds resulted from the treatment, as though the regenerative capacity of the gemmae had decreased (Fig. 8 A-C). The buds arose as cylindrical, succulent structures, often unfolding into " cups " or " feet ".

When gemmae were grown for 15 days before removal of growing points, the results were strikingly different from those grown for 5- and 10-day periods. No buds at all arose on the cut edges of the gemmae, but numerous small buds developed on their upper surfaces accompanied by formation of rhizoids (Fig. 9 A-C).

If gemmae were grown for one month before excision of the growing points, some regenerative capacity was still displayed. Regeneration was of the same type as for the gemmae treated after only 15 days' growth. No buds were formed on the cut edges, but developed on both upper and lower surfaces of the gemmae, and these grew out into upright lobes (Fig. 10 A-C).

Destruction of Growing Points by Burning — The growing points of numerous gemmae were destroyed by scorching them with a hot needle. However, the gemmae were so minute that the hot needle, though small, destroyed much more than the growing points. In some gemmae less than half the tissue remained alive and green with extensive dead tissue around the growing points and even around the whole peripheries of the gemmae. New buds developed from both upper and lower surfaces of the small living green areas (Fig. 11 A-G).

Crushing by Rolling — Gemmae were rolled with a wooden roller with varying pressures but not with pressures heavy enough to mangle the tissues. In some, the auricle of a gemma produced a lobe with a bud developing between the lobes in nearly all the examples (Fig. 12 A-C). In others (Fig. 12 D) new thalli grew out from the under surfaces of auricle lobes. Some of the gemmae showed abundant growth of rhizoids from the upper surfaces (Fig. 12 A, D).

Sandpapering of Surfaces — The surfaces of gemmae were lightly stroked with fine sandpaper. As a result, the auricle lobes of many grew out into rounded form and nearly all formed cylindrical lobes on these auricles, developing mostly from the under surfaces (Fig. 13 A-D). In certain others, which perhaps were treated somewhat more severely, the gemmae grew little and showed marked succulence of the growing ends. Succulent thalli started out from the growing points and many of these showed immediate splitting (Fig. 14 A-C).

Slicing off Surface of Gemmae — The upper surfaces of central portions of gemmae were sliced off with a razor blade. The central portions died, leaving only a peripheral band of living tissue. From this band, very numerous buds arose (Fig. 15 A, B). This experiment, like that of puncturing, resulted in gemmae with a hole in the middle, yet the results are very different. Possibly the difference was caused by the death of the central tissue *in situ* with a resulting production of some bud-stimulating substance or substances.

Effect of Gases

Nitrogen — Gemmae were placed on wet filter paper in chambers from which

FIGS. 7-15 — Effects of different kinds of injury on gemmae growth. Fig. 7 *A-C*. Removal of growing points on gemmae grown for 5 days; observe formation of numerous buds from cut ends. × 14. Fig. 8 *A-C*. Same as above, on gemmae grown for ten days; regenerative capacity has dwindled as compared to that on 5-day old gemmae. × 14. Fig. 9 *A-C*. Same, on gemmae grown for 15 days. Regeneration from body of gemmae instead of from cut ends as in Figs. 7 and 8 is observed. × 14. Fig. 10 *A-C*. Same, on 1-month old gemmae; regenerative capacity is still retained. × 14. Fig. 11 *A-G*. Destruction of growing points by scorching (unstippled portion indicates dead tissue); regeneration is observed from green surfaces within two weeks. × 14. Fig. 12 *A-D*. Crushing by rolling (with a wooden roller); auricles develop into thalli (*A, B*), buds develop between auricles (*A-C*) and new tips from under surface of auricles (*D*). × 14. Fig. 13 *A-D*. Sandpapering of surfaces. × 14. Fig. 14 *A-C*. Same as above; growing points with split tips (*A, B*) and development of cushion-like tissue at the apical notch (*C*) are observed. × 14. Fig. 15 *A, B*. Effect of slicing off middle portion; regeneration occurs from periphery and bizarre shapes are formed. × 22.

the air was driven out by nitrogen gas from a tank. After the lapse of different periods of time—1 day, 2 days, etc.,— the gemmae were grown on moist filter paper in petri dishes for two weeks. Treatment for 1 day was sufficient to show marked results. Growth was nearly normal (Fig. 16 A), but the gemmae were rather pale

green with bright green tips. Rhizoids were common on the upper surfaces, as were also split tips.

After two days in nitrogen the thalli developed much as those with one-day exposure. However, the thalli showed more inhibition of growth, greater succulence, and more splitting, perhaps as a result of the increased succulence. Rhizoids were abundant on the upper surfaces.

After 3 days' exposure, the gemmae developed almost as after only 2 days in nitrogen. After 4 days in nitrogen the gemmae became somewhat more elongated, some were very succulent. Spherical upright buds appeared on the gemmae themselves or on the young thalli. These buds developed into " feet " directly on the thalli or on the gemmae without any " legs " or stalks at all (Fig. 16 B-D). The rhizoids on the upper surfaces were less numerous than on gemmae treated for 2 or 3 days. Five days in nitrogen produced no noticeable change from 3-day or 4-day exposures except that the rhizoids were further decreased in number.

Nitrogen Gas Bubbled through Water — Gemmae were floated in water through which nitrogen gas was bubbled in a slow stream in a closed chamber. The gemmae were thus washed in water through which nitrogen was passing continuously. These gemmae were subjected to this treatment for periods of 1 day, 2 days, etc., up to 7 days. They were grown on filter paper in petri dishes for two weeks and then examined.

One-day treatment resulted in lobes mostly grown out. Rhizoids were seen on upper surfaces of some.

Treatments for 2 or 3 days showed little difference from the 1-day treatment. The thalli stood upright. On the set which had been treated for four days, splitting was frequent. Fig. 17 A shows a gemma which has formed two thalli, both of which have split immediately after formation. In both of these thalli the lower lobes predominated. In Fig. 17 B the upper lobes were most strongly developed.

After 7 days of treatment the development was much the same as after four days of treatment except that the thalli were narrower and perhaps more succulent. They still stood upright.

Treatments with CO_2

Gemmae were kept in chambers filled with CO_2 for varying lengths of time. They were then grown for two weeks on wet filter paper.

Exposure to CO_2 for 1 Day — Some had splits which were mostly outgrown. Growth in general was considerably retarded. Rhizoids appeared on upper surfaces but were few in number.

Exposure to CO_2 for 2 Days — These were more succulent and had more rhizoids than those resulting from 1-day treatment. Splitting was common. Extra lobes, buds and fringed margins were common.

Exposure to CO_2 for 3 Days — Many gemmae were completely dead and none had grown much. Growing points were dead but parts of some gemmae remained alive. Many buds were formed on upper and lower surfaces of the gemmae (Fig. 18 A-C).

Exposure to CO_2 for 4 Days — All the gemmae were killed by this treatment.

Effect of Bubbling CO_2 — The gemmae, in this treatment, were immersed in White's solution through which CO_2 was bubbled continuously. The liquid was overlaid by an atmosphere of CO_2 so the gemmae were not only kept from air but were subjected to the acid solution produced by the CO_2 which had dissolved in the liquid. The liquid showed a pH of 4·2 at the end of this period. After treatment, the gemmae were grown in the usual way and examined after the lapse of two weeks.

Gemmae given this treatment for 1 day were seriously affected. Adventitious thalli developed from upper and lower surfaces of the gemmae. Frequently each auricle of a gemma bore an upright bud.

After 2 days of treatment the growth of the gemmae was much retarded and they became very succulent.

A 3-day treatment produced brown and dead patches in the gemmae which showed very slow growth and pronounced succulence. Cylindrical and succulent buds

formed all over the upper and lower surfaces of the gemmae (Fig. 18 D, E).

Treatments with Oxygen — Gemmae were placed in chambers in which the air was completely replaced by oxygen from a tank.

Six hours of exposure produced rather marked effects. Some splitting occurred and rhizoids were abundant on the upper surfaces.

After 1 day's exposure to oxygen, eighty per cent of the thalli which grew from the growing points were split (Fig. 19). No rhizoids grew from the upper surfaces.

Two days' treatment with oxygen produced more vigorous growth than that for 1 day.

Thalli grown from gemmae subjected to 3 days in oxygen showed numerous upright or nearly upright lobes.

Exposures of gemmae for periods longer than 3 days led to the formation of narrower and narrower thalli as the lengths of exposure increased. Fig. 20 A, B shows the long, narrow split thalli produced by gemmae which had had 7 days' exposure. Gemmae were still alive and green after ten days in oxygen and gave rise to thalli which were still narrower than those shown in Fig. 20 B.

Immersion of Gemmae in Water through which Oxygen was Bubbled Continuously — One day of this treatment led to a splitting of nearly all of the thallus lobes which grew from the treated gemmae. In these splits nearly all the lobes were turned back and grown out equally. This was very unusual, for regularly either the upper or the lower lobe of a split predominates and more often than otherwise it is the lowermost lobe which becomes dominant. Rhizoids were absent from the upper surfaces of these gemmae.

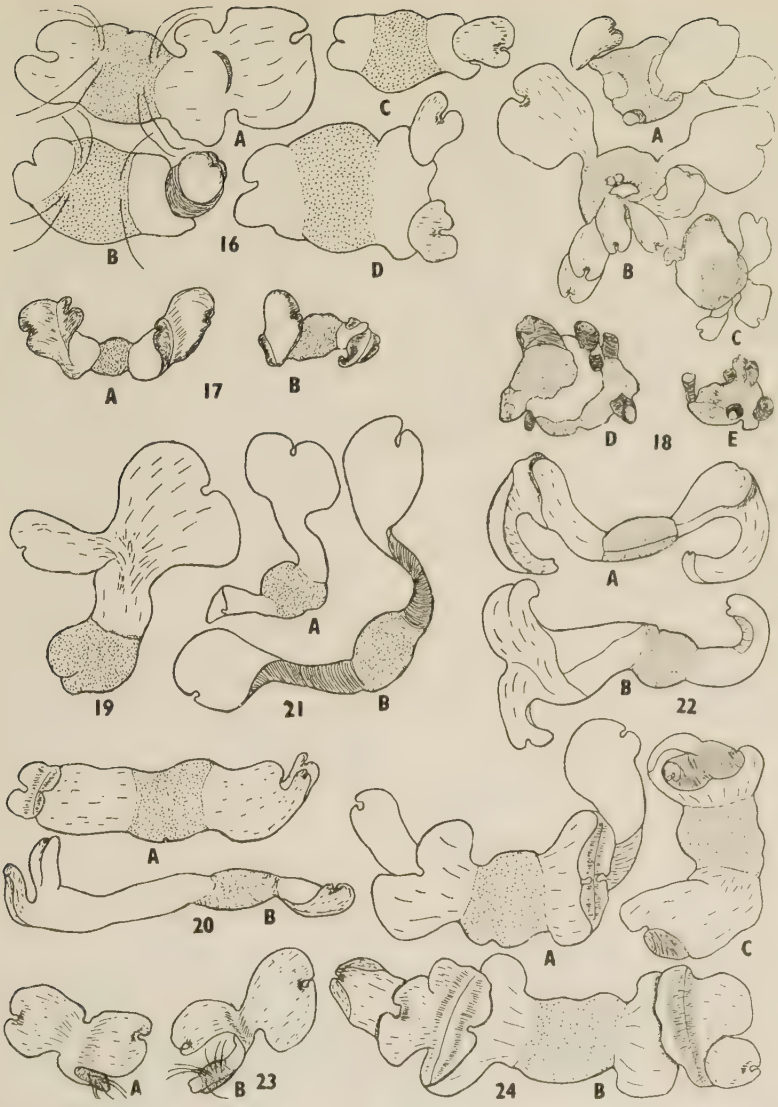
Gemmae which had been treated for 2 days showed less splitting and a great lengthening and narrowing of the thallus lobes which grew out. After 3 days of treatment, rhizoids developed abundantly on the upper surfaces of the gemmae and the thalli grew longer and narrower. When gemmae were grown after 7 days' treatment they formed long, narrow, thickened, stalk-like structures which spread out abruptly at the tips (Fig. 21 A, B).

Growth of Gemmae in White's Solution on a Shaker — The gemmae were floated in White's solution in flasks and continuously shaken, with 80 excursions per minute. After two weeks the gemmae showed practically no growth. Thalli had grown out from the growing points which were succulent and considerably thickened. These were narrower and shorter than normal.

Effect of Immersing Gemmae in Water through which Air was Bubbled Continuously — Gemmae were immersed in White's solution in an upright cylinder. A current of air was bubbled through the water at a speed sufficient to keep the gemmae agitated. At the end of a week the thalli were found to have grown out long and thin and bent into loops at the ends. Many of the thalli were succulent and had split at the tips (Fig. 22 A, B).

The Effects of Sudden Changes of Air Pressure — In this test gemmae were enclosed in a metal chamber provided with a valve through which air was admitted from the air line of a gasoline station. The maximum pressure available was 55 lb. per square inch. When the chamber was pumped full of air at the maximum pressure, a plug was loosened and the air rushed out with explosive force. A succession of 10 inflations and releases had a pronounced effect. The thalli became very succulent and most of them were split (Fig. 23 A, B). The second growing point was inhibited. Rhizoids were conspicuous on the upper surfaces of the gemmae. Five such releases had little effect save to induce some rhizoids on the upper surfaces, whereas the subjects of a single compression and release, like the controls, showed no rhizoids at all.

Twenty compressions and releases showed an intensification of the results of ten such treatments. Splitting was very common. New thalli grew out from the apical notches, arising from the under surfaces of the older thalli (Fig. 24 A, B). Definite spurts of growth took place (Fig. 24 B). As many as 4 such successive growths were observed in some of the plants. Many gemmae became succulent and produced cylindrical or spherical upright buds between the auricles (Fig. 24 C).



FIGS. 16-24 — Effects of various gases. Fig. 16 *A-D*. Effect of gaseous nitrogen. × 15. Fig. 17 *A*, *B*. Nitrogen gas bubbled through suspension of gemmae. × 7. Fig. 18 *A-E*. Treatment with CO_2; *A-C*, gaseous CO_2; *D*, *E*, CO_2 bubbled. × 15. Fig. 19. Treatment with oxygen for 1 day. × 15. Fig. 20 *A*, *B*. Same, for 7 days. × 15. Fig. 21 *A*, *B*. Same, bubbled. × 15. Fig. 22 *A*, *B*. Effect of immersing gemmae in water through which air was bubbled continuously. × 15. Fig. 23 *A*, *B*. Effect of sudden releases of air pressure — 10 releases. × 7. Fig. 24 *A-C*. Same, 20 releases. × 15.

THE EFFECT OF DIRECT PRESSURE ON GEMMAE — Gemmae were placed between two metal plates with plain, polished surfaces. Varying weights were applied to the upper plate in different trials.

Weights ranging from 50 to 1,500 grams did not produce rupture of the tissues, but even a weight of 50 grams was not without effect.

Gemmae which had been subjected to weights of 100, 250, 350 and 1,500 grams were healthy and well grown 10 days after treatment, but they all showed 5 or 6 extra lobes.

A weight of 2,000 grams caused rupture of the gemmae in the central regions and the extra lobes were reduced in number. The thalli grown from these gemmae were less vigorous than those previously mentioned. Ruptured gemmae became fleshy and formed vertical lobes or cylindrical buds from the auricles (Fig. 25 A).

Gemmae subjected to a weight of 4,000 and 5,000 grams gave identical results. The two auricles at each growing point of a gemma each formed a thick, fleshy thallus, often showing splitting and mostly outgrown. These thalli showed a marked tendency to asymmetrical growth so that the apical notches became displaced towards the sides of the thalli (Fig. 25 B-D).

Effect of Plasmolysis and Subsequent Deplasmolysis of Gemmae — A 10 per cent solution of the plasmolysing agent was used. The time required for plasmolysis without rupture of the protoplasmic membrane was determined by watching under the microscope. Gemmae were then transferred to a petri dish containing White's solution. Observations were made every week for morphological changes, if any, during growth owing to treatment with the hypertonic solution.

NaH_2PO_4, 10 per cent — In one week the auricle lobes grew out and were widely divergent, disposed at right angles to the longitudinal axes of the gemmae (Fig. 26 A-C). The lobes possessed fringed margins. Spherical buds developed at the growing points between the auricles accompanied by rhizoid formation (Fig. 26 C-G). These buds never flattened out into normal thalli even after growth for 4 weeks. They appeared swollen and thickened into a pointed conical tip and never developed thalli until after the decay of the gemma body.

In another set of gemmae, buds failed to develop at the growing points indicating inhibition. The gemmae became succulent and several adventitious buds arose on their surfaces (Fig. 26 H-K) in over a month's time. No rhizoids were formed on either of the surfaces of the gemmae or near the buds.

Gemmae plasmolysed for 12 minutes were mostly dead.

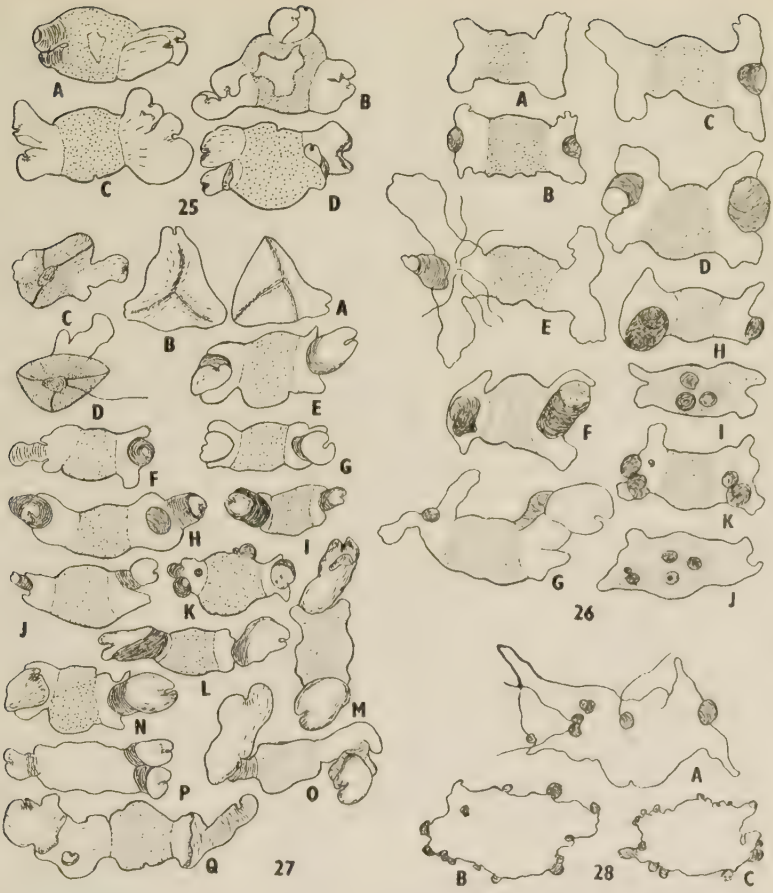
NaCl, 20 per cent — Considerable morphogenetic variations were evident in gemmae subjected to the plasmolysing effect of the solution. Many were killed. Others had live growing points. A few assumed a pyramid-like shape, having become succulent and swollen with a flat base (Fig. 27 A-D). Not many rhizoids were observed on the surface. Grown over a period of four weeks still showed the gemmae to be quiescent. The primordia of buds were apparent at the growing points. The new tips, however, were sluggish and thickened.

Growth was to a considerable extent inhibited in most gemmae, especially of the buds that had formed between the auricle lobes (Fig. 27 E-J), even after cultivation for a month. In still others, a few adventitious buds were formed on the surfaces of the gemmae (Fig. 27 K).

Normal apical growth was observed in those apparently less affected, but the thalli were unequally lobed at the apical notch and thickened, followed by splitting.

Gemmae were grown in a solution of NaCl and White's mineral elements. A concentration of 0·5 per cent of the salt showed succulence of the new tips from the growing points. Splitting was common. Rhizoids were abundant.

Culture in a 1 per cent NaCl solution showed the gemmae to be retarded in growth in two weeks. Some were killed. Those that were alive were transferred to White's solution alone without the sodium salt. Succulent, cylindrical and spherical buds were formed at the growing points, which later developed into shallow, peltate cups or " feet " at the top (Fig. 27 L-P). In some the stalks were so short as to make the thalli appear to rise directly on the gemmae. Occasionally, side buds originated from the " feet "



FIGS. 25-28 — Effects of direct pressure and plasmolysis. Fig. 25 *A-D*. Five kilograms pressure (unstippled portion denotes dead tissue); cylindrical lobes (*A*), auricles developing new tips (*B-C*) and splitting (*D*) are shown. × 15. Fig. 26 *A-K*. Effect of plasmolysis with 10 per cent NaH_2PO_4 solution for 5 minutes and grown normally for 4 weeks. Auricles are broadened, fringed, and reflexed backwards (*A-E*) and succulent spherical buds develop between auricles; *I-K*, gemmae grown for 4 weeks on 5 per cent solution of NaH_2PO_4 and transferred on to White's solution; inhibition of normal growth and adventitious buds developing from bodies of gemmae are shown. × 15. Fig. 27 *A-Q*. Effect of plasmolysis with a solution of 20 per cent NaCl; *A-D*, pyramidal shape of gemmae and growth from one of the growing points; *E-K*, spherical and cylindrical buds formed between auricles or on body of gemma, one month after treatment; *L-Q*, later stage, showing expanding top of cylindrical buds appearing as " feet " or " cups " or irregular split thalli. × 22. Fig. 28 *A-C*. Plasmolysis with 10 per cent solution of KNO_3 and grown on 2, 4-D, 0·1 per cent for 10 days. × 15.

(Fig. 27 M-Q). Some showed splitting, the splits mostly grown out (Fig. 27 Q).

In 2 per cent NaCl the gemmae were all killed, although in a few the growing points were still alive.

KNO_3, 10 per cent — Numerous adventitious thalli developed from both surfaces of the gemmae. More than a dozen could be counted. They were of varying sizes, shapes and degrees of growth. They originated as cylindrical, button-like structures which grew out as normal thalli. Rhizoids were also abundant. Apical growth was considerably reduced.

Plasmolysis with 10 per cent KNO_3 followed by growth on 2, 4-D (0·1 per cent) produced gemmae of large size

and very irregular growth. Very numerous buds were developed, mostly on the margins, but these remained inhibited (Fig. 28 A-C).

Plasmolysis with a 10 per cent solution of $Ca(NO_3)_2$ gave results which were very similar to those obtained by the use of KNO_3.

The Effects of Ultraviolet Radiation of Gemmae — Gemmae were placed flat on filter paper wet with White's solution in petri dishes. The dishes were set 27·5 cm below a 30 watt General Electric germicidal lamp, dish covers were removed, and the gemmae were exposed for periods of time ranging from 5 to 80 minutes. The petri dish covers were replaced and the dishes removed to the greenhouse where the gemmae were grown for 2 weeks before examination.

Irradiation for 5 minutes produced an abundant crop of rhizoids on the upper surfaces of the gemmae, mostly towards the growing points. The thalli had irregular tips, an unusual number of which showed irregular splitting (Fig. 29 A-C). Many others showed widely divergent tips (Fig. 29 D). The bodies of gemmae were elongated and expanded (Fig. 29 A, D, E). The auricles grew into flat, cylindrical, twisted structures (Fig. 29 D, E).

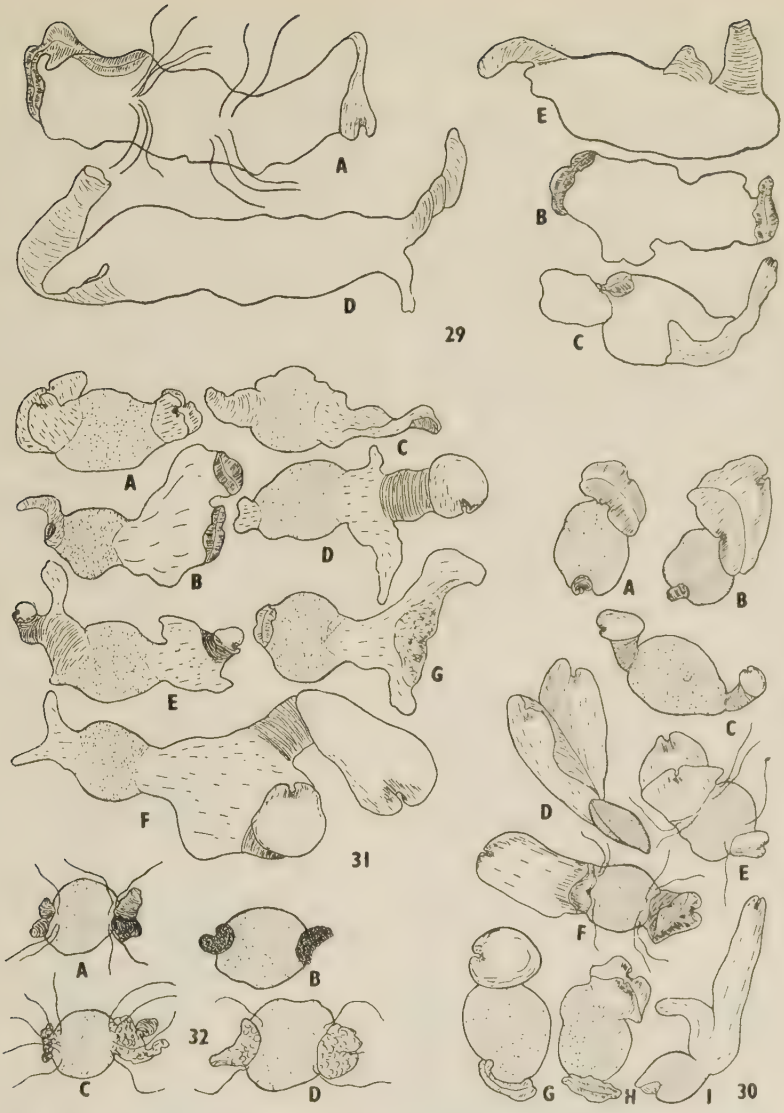
Ten minutes of irradiation slowed down growth of the gemmae. More rhizoids appeared than on the gemmae which had only 5 minutes of the treatment. Most of these rhizoids were grouped at the ends of the gemmae near the growing points but some were scattered over the upper surfaces. Eighty per cent of the thalli developed split tips and grew upright about 1 cm above the filter paper. The lobes of the split thalli were turned back horizontally and grown almost equally (Fig. 30 A-I).

The general effect given by viewing a group of these in a petri dish was that of a number of embryos with semicircular cotyledons spread out horizontally (Fig. 30 A, B). To be sure, there was considerable variation to be seen since some lobes were narrow and long, while others were short and wide. On some thalli the lobes were rather unequal; some had rounded ends, whereas others had strongly notched ends, and in still others, the splits had grown out; the upper lobe was seen only as ligular vestiges. In a few, one of the growing points was inhibited.

The gemmae subjected to 20 minutes of ultraviolet radiation showed considerable morphological peculiarities (Fig. 31 A-G). Splitting as for 10 minutes of treatment was common. The thalli which grew out appeared as cylindrical upright buds which split at the tips. But instead of producing a predominance of equally grown lobes, almost all of these showed unequal growth of the lobes. Some of the upright cylindrical lobes produced buds on their sides. Rhizoids were seen on the upper surfaces but in much smaller numbers than on gemmae treated for 5 minutes or for 10 minutes. Some showed no development of the auricle-lobes but the growing points developed into cylindrical, elongated structures with pointed tips showing no differentiation (Fig. 31 C). In others the auricle-lobes were prominent and buds were formed between them, developing into peltate "cups" or "feet" (Fig. 31 D-F). Occasionally, one came across a gemma which had developed a very widened thallus with cushion-like apical notch with warty outgrowths (Fig. 31 G).

An ultraviolet treatment for 40 minutes caused the death of nearly all the treated gemmae, as evidenced by the loss of chlorophyll and brown colouration on the surface. However, the growing points remained alive and bright green, and each formed 1-3 cylindrical buds (Fig. 32 A). Some of the living gemmae were faintly green, others very pale. Undifferentiated, callus-like masses were formed from the growing points (Fig. 32 B-D). Rhizoids were very profuse on these newly formed structures. Warty excrescences were also abundant on the new callus-like mass of tissue. The latter often grew into bizarre horn-like shapes (Fig. 33 A-D). Some of these bore spherical, knob-like branches (Fig. 33 A, C, D, *br*). These knobs later developed into "cups" or "feet", borne on cylindrical stalks (Fig. 33 E).

An exposure of 50 minutes to the ultraviolet light showed similar features as for 40 minutes, though growth was a little more retarded for the same period.



FIGS. 29-32 — Effects of ultraviolet radiation on gemmae. Fig. 29 *A-E*. Exposed for 5 minutes. Fig. 30 *A-I*. Exposed for 10 minutes. Fig. 31 *A-G*. Exposed for 20 minutes. Fig. 32. Exposed for 40 minutes. All × 15. (Description in text.)

The lobes which grew out were almost all upright and cylindrical, with tips which split in such a way as to form " cups " or " feet " instead of split lobes of the double-decker type (Fig. 34 A-D). Some upright lobes showed irregular branching and numerous bizarre shapes.

After 60 minutes of ultraviolet light, the gemmae became distinctly brown, except for a few which showed some green spots in them but did not enlarge at all. Cylindrical and spherical buds developed at the growing points which were still alive (Fig. 35 A-E). These

remained dormant and showed no further growth indicating inhibition. This is in marked contrast to those exposed for only 50 minutes.

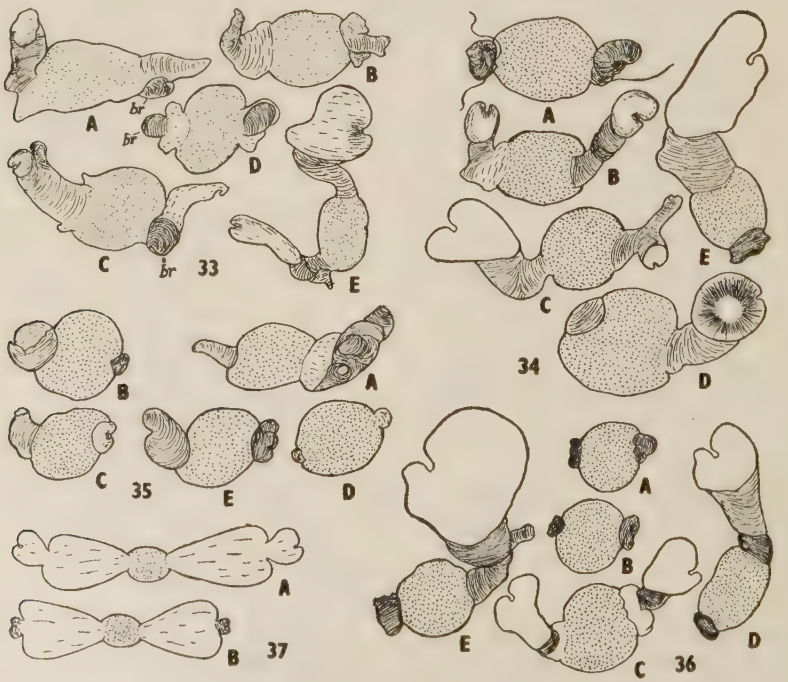
Gemmae subjected to 80 minutes of ultraviolet light were nearly all killed, and were very pale. In a few, although the interior of the gemmae showed no green tissue, the growing points were still alive. From these growing points tiny succulent lobes grew out (Fig. 36 A, B). Although inhibited in growth when grown in White's solution for a month, they developed into surprisingly large, upright cylindrical stalks with very large flat tips (Fig. 36 C-E).

The Effects of Ultraviolet Light on Gemmae which had been Grown for Two Weeks before Treatment — Gemmae were put on filter paper in petri dishes and were grown in a greenhouse for two weeks before treatment. During the two weeks, thalli had grown out from all the growing points so that each gemma had a triangular thallus at each end. These gemmae with their attached thalli were given ultraviolet radiation of the same intensity as the ungrown gemmae had received.

Treatment for 5 minutes caused an abrupt cessation of growth of the thalli, which was followed by the formation of a new and much narrower but thicker thallus from *each* apical notch (Fig. 37 A). The gemmae remained green.

After 10 minutes irradiation the thalli turned brown and appeared to be killed. But from each apical notch a new, smaller, and much thicker thallus arose (Fig. 37 B). In this set also the gemmae remained green.

After 20 minutes treatment the thalli became very brown and appeared to be dead, although the gemmae remained green. Later some apical notches on the apparently dead thalli formed very small beginnings of new thalli.



FIGS. 33-37 — Effects of ultraviolet radiation on gemmae. (*br*, branch). (Description in text). Fig. 33 *A-E*. Exposed for 40 minutes. × 15. Fig. 34 *A-E*. Exposed for 50 minutes. × 42. Fig. 35 *A-E*. Exposed for 60 minutes. × 15. Fig. 36 *A-E*. Exposed for 80 minutes. × 15. Fig. 37 *A*, *B*. Same as above, of gemmae already grown for two weeks before treatments; exposed for 5 minutes and 10 minutes respectively. (Semi-diagrammatic). × 15.

A treatment for 40 minutes resulted in the death of all thalli and their parent gemmae.

THE EFFECTS OF GAMMA RADIATION FROM A COBALT 60 SOURCE — Gemmae were placed on wet filter paper and then into closed vials. They were then exposed to dosages of gamma rays ranging from 1,000 to 80,000 R. They were then transferred to petri dishes and grown for 3 weeks.

1,000 *R* — The gemmae as compared to controls showed delayed growth. Rhizoids were common on the upper surfaces. The lobing was somewhat irregular and many of the lobes were thickened. Some showed split tips.

2,000 *R* — Rhizoids as for 1,000 R. Some splitting was present and others had very irregular lobes. Many of the gemmae showed three thalli per gemma and also the ends of the thalli were abruptly widened.

3,000 *R* — About as for 2,000 R but the lobes were thickened and growth more delayed.

4,000 *R* — Growth was more delayed than for 3,000 R and the upright thick lobes were more irregular. The rhizoids developed as for 2,000 R and 3,000 R.

5,000 *R* — Growth was scarcely different from that after 4,000 R treatment but it was more irregular.

10,000 *R* — The gemmae were thickened and only slightly elongated. The rhizoids developed as for all the preceding dosages. The beginnings of the upright lobes were seen in some of the notches.

40,000 *R* — Growth of the gemmae was almost completely inhibited. Some showed slight elongation at the notches of the gemmae.

80,000 *R* — All of the gemmae died without growth.

Discussion

We have found, and, in a somewhat imperfect fashion, have shown a great number of morphological variations which have been induced by the action of various physical factors. We have no hope of explaining the minor variations, but we think the major morphological types may be attributed to wounding since practically all the treatments used must have resulted in wounding in some way and to some degree. Furthermore, we think the wounding has not given rise to stimulation but has had the effect of interrupting the apical influences. One of the apical influences is the inhibition of the formation of adventitious buds as Dickson (1932) clearly showed. But it is possible that there is another apical influence which governs the growth of gemmae in general. This might be, for instance, an auxin concentration, much lower than that required for bud inhibition, but one which would induce continued growth of the thallus.

The most frequent response, indeed, following nearly all the treatments is the development of adventitious buds. We believe this to be due to the removal of the inhibition of the apical buds rather than to any type of wounding. One of our experiments gives strong evidence of this. The perforation of gemmae with a needle causes extensive wounds, but the original buds at either end of each gemma grow out without the formation of any adventitious buds whatever (Fig. 3). More extensive wounding (Fig. 4 A) still produced no adventitious buds. But if cuts were made so that the outer portions of the gemmae were separated and only the inner extensions of the segments remained attached, the outer ends of these segments formed adventitious buds (Fig. 4 B).

The more complete the removal of the effect of apical buds, the larger is the number of adventitious buds produced (Figs. 18, 26, 28). This seems to show why very small segments of gemmae and thalli will form buds (Fig. 1 E). Voechting (1885) showed this long ago for thalli of *Marchantia*. The division of the gemmae into still smaller segments by cutting caused death of the small parts, but isolation by other means leads to formation of almost innumerable buds (Fig. 32 C, D). This effect was brought about by ultraviolet light, but we have seen similar developments following plasmolysis of gemmae as Nagai (1919) has reported. Dickson (1932) found bud formation similar to this in his control cultures but gave no explanation of them.

We (1955) have found that a similar abundance of buds may be produced by chemical treatments.

Nagai was of the opinion that the formation of adventitious buds is a direct consequence of stimuli caused by the change of " structure " of the protoplasm and not the result of mere mechanical injury. Our observations lead us to think that even pulling apart the protoplasmic membrane from the cell wall (though still connected by a number of fine protoplasmic fibriles between the wall and the contracted mass as Nagai suggests) is one form of injury enough to cause disturbance of the inner co-ordination, and thus an interruption of any dominance. Then any cells or small group of cells could become meristematic and develop a bud. How this effect can be produced by ultraviolet light we have no idea, but we believe the phenomenon in general is related to the formation of a callus. Callus formation might be brought about by a more complete removal of inhibitions with the subsequent independent growth of almost every cell.

We have, in fact, seen an approach to callus formation in gemmae. We hope to make a further study of this problem.

Our next concern is with the manner of growth of the buds, original or adventitious, when they begin to grow out. The original buds, if alive, have overcome their inhibition, whatever its cause and nature, and begin to grow. Likewise, the newly formed adventitious buds continue development. A few of these buds, original or adventitious, at once produce normal thalli (Figs. 3 A, 6 A). Some otherwise normal thalli show a greater growth of one lobe than in the other (Fig. 25 A-D). It seems that all buds of whatever origin and regardless of the type of treatment to which they have been subjected, eventually form normal thalli. We have never seen, in our studies, any evidence of the induction of a mutation, or any permanent morphological variant. But in the process of recovery of the original form, these structures undergo some " sea changes " and, for a time, take on remarkable forms which we are as yet incapable of explaining. One of the most remarkable of these we (1954, 1955) have called " splitting ". It was first reported by Förster (1927) and studied anatomically by Halbsguth (1936). Instead of a tip producing two lobes which lie side by side, the two lobes lie one above the other (Fig. 14 A, B). What really happens is that the plane of division of the apical cell is rotated through an arc of 90 degrees. Although we have never seen splitting in undisturbed plants growing in the greenhouse or in the field, it may be induced by the most diverse chemical and physical factors. We have no explanation whatever of the changed plane of the apical cells.

After splitting, the two lobes thus formed seldom grow parallel to each other. The lower lobe usually outgrows the upper (Figs. 6 J and 30, F). Sometimes the original thallus grows upright and the two lobes spread out horizontally (Fig. 23 A, B). Splitting appears to follow increased succulence and thickening of the thallus tips. It may be that splitting is a result of a reduced rate of expansion and a resultant thickening of the thallus tips.

There is variation in placing of the adventitious buds. They may occur on the upper surfaces only, on the lower surfaces only, or on both upper and lower surfaces of the gemmae. Buds sometimes have been found growing out from the cut ends of the gemmae. The gemmae shown in Fig. 15 obviously could form buds only on their margins, but those shown in Fig. 28 showed a preference for a marginal location of the buds.

Many of the buds, both original and adventitious, showed disturbed patterns of growth and orientation. Instead of forming normal thalli they produced spherical or cylindrical outgrowths. They were usually directed upward instead of in a normal horizontal plane (Figs. 9, 10, 16, 18, 26, 27). After a certain amount of growth the spherical buds usually grew into cylindrical structures, and the cylindrical structures apparently began to approach normal growth again. The upper ends of these began to flatten out in a horizontal plane and form thalli (Figs. 8, 10, 11, 27, 33, 34, 36). These curious formations we facetiously referred

to as " feet ". It may be assumed that the thickness of the upright cylindrical stalk had an effect on the shape of the thallus on its top. No explanation, however, is available of the formation of thalli with practically no upright stalk (Fig. 16 C, D).

Thus far we have considered the growth and development of buds from the gemmae. There is considerable variation in growth and differentiation of the gemmae themselves. They vary in size and shape, in outlines, and in rhizoid formation. Our camera lucida drawings, in fact, are unable to express many of the subtle differences apparent to one who views them under the wide-field microscope.

Variations in succulence are great. Perhaps the extreme succulence is shown in the pyramids illustrated in Fig. 27 A-D. Great succulence would be expected to produce a sphere, and many buds have formed such spheres. It is difficult to see what growth factors produce three-sided pyramids with such flat planes and sharp edges as one sees here.

Rhizoid formation, too, was seen to vary greatly with varying types of treatment and even with varying intensities of a given treatment. Thus far we are unable to develop an explanation of their formation.

Our data give us no indication at all as to whether the growth and development of the gemmae like the development of adventitious buds may be due to the elimination of inhibitions. All these treatments presumably have caused injuries, and these injuries have resulted in disturbed growth patterns. The variations as shown are considerable, and as has been said, others have escaped description or delineation. Much more study will be needed before these patterns can be explained.

Summary

1. Gemmae of *Lunularia cruciata* have been removed from the cups in which they grew, treated with various physical agents, and then grown on moist filter paper in petri dishes in a greenhouse to observe their growth forms.

2. The agents employed were centrifugation, vacuum, slicing, perforation, excision of growing parts, crushing by rolling, crushing by weights, abrading of surfaces, exposure to oxygen, nitrogen, and carbon dioxide, compression and sudden release of air, treatment with ultraviolet rays and with gamma rays from a sixty cobalt source, and plasmolysis. Gemmae were also grown in a series of solutions with varying hydrogen-ion concentrations.

3. The treatments resulted in a variety of growth forms, many of which are illustrated by camera lucida drawings.

4. One of the most usual results of the treatments was the formation of adventitious buds. This is interpreted as a result of the removal of apical inhibitions.

5. All growth changes appeared to be temporary. No mutations have been found and all the growth types show a strong tendency to revert to the normal as quickly as possible.

6. The buds, both original and adventitious, show considerable variation in the forms into which they develop, and the regions of the gemmae on which they form. Many show disturbed geotropic relations and grow upright. These upright growths show a tendency to form cylindrical upright stalks and later to begin formation of thalli on their upper ends.

7. The gemmae themselves, when grown, show much variation in size, outline, succulence and rhizoid production. The extreme of succulence was reached in the production of a three-sided pyramid with flat planes and sharp edges.

8. The formation of adventitious buds appears to result from the removal by any agency of apical inhibitions.

9. Only two treatments were without effect. Centrifugation produced no result. Hydrogen-ion concentrations below 3 and above 11 were lethal, but between 3 and 11 not much difference in growth could be seen.

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SOME RECENT ADVANCES IN MORPHOLOGICAL PALAEOBOTANY*

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It seems desirable to state at the outset the limitations that we have chosen in preparing this discussion, which, to begin with, is undoubtedly coloured by our own interests in palaeobotany. These interests are principally morphological and the discussion, being prepared for a morphological journal, seems to be justified in this respect. A great many contributions have been made in palaeobotany during the past 15 to 20 years and in certain countries the number of workers and their breadth of interest have expanded considerably. We have made no attempt to include certain areas of palaeobotanical investigation, which are now rather clearly defined subdivisions of the science and which would require treatment by specialists to be significant as critical reviews. Thus we have purposely excluded discussions of spore and pollen investigations, Tertiary plant studies, studies of fossil woods, and purely floristic or stratigraphic works; and our treatment of the algae is very sketchy.

The selection of a time limit has proved to be even more difficult than subject material. What constitutes a "recent advance" is dependent on several factors, including the reader's familiarity with the field and the number and nature of textbooks reviews that have been written. We have, in general, tried to cover investigations of the past decade principally for the non-palaeobotanist, but in numerous instances we have brought in studies that antedate that period where it seemed desirable to bring out the significance of more recent contributions.

Within our chosen area of morphological and evolutionary studies it should be obvious that in the space available our treatment can be in no way complete. It is hoped that our presentation will serve

*Publication authorized by the Director, U.S. Geological Survey.

to point out certain highly significant contributions and will lead the interested reader to a more detailed perusal of the literature. We would also mention that we claim to have contributed nothing that is original, with the exception of a few criticisms and comments on apparent evolutionary sequences in the vascular plants. We have necessarily drawn freely from the works of numerous authors and if unacknowledged phrases appear in our text it is unintentional, full credit being due to the original investigator concerned.

General Works of Particular Interest

Several excellent text-books have been published in very recent years. In 1950 Kräusel's "Versunkene Floren" appeared, a small text of 150 pages accompanied by 64 pages of plates. In 1953 a second edition of Walton's "An introduction to the study of fossil plants" appeared bringing up to date his original edition of 1940. An especially noteworthy text is Magdefrau's (1953a), "Paläobiologie der Pflanzen" a book of 438 pages, thoroughly up to date and beautifully illustrated, covering essentially the entire field of palaeobotany in exceptionally well-selected fashion. Magdefrau's reviews (1953b, 1954) in "Fortschritte der Botanik" also deserve mention as valuable summaries of recent work in palaeobotany, as well as his "Die Geschichte der Pflanzen", a 38 page account of palaeobotany that appeared in "Die Evolution der Organismen"; also deserving of mention is his "Vegetationsbilder der Vorzeit", which includes 18 plates of landscape restorations. Corsin (Bertrand & Corsin, 1950) has recently prepared a short text to accompany Bertrand's now rather well-known seven plates of Devonian, Carboniferous, and Mesozoic landscape restorations. In 1949 Jongmans issued his "Het Wisselend Aspect ven het Bos in de Oudere Geologische Formaties", a book of more than 160 pages, which might be described as a "text-book of fossil forests". For the teaching palaeobotanist it is, being profusely illustrated, an especially useful account of fossil forests. This is a really unique volume in palaeobotany, and it is to be hoped that it may someday be translated into other languages.

Contributions to Our Knowledge of the Lower Plant Groups

Although, as mentioned above, we have made no attempt to survey carefully the recent literature dealing with the fossil algae, a few accounts may be mentioned that are of particular interest. Botanists and geologists who are concerned with fossil algae and particularly the rock building groups will welcome Johnson's (1954) "An introduction to the study of rock building algae and algal limestones". This is a timely and significant compilation of the algae that are concerned with rock building. It includes a systematic consideration of the various groups concerned as well as the general ecological relationships of these plants.

In a paper that will, among other things, stimulate thought concerning the ultimate origin of plant life Tyler & Barghoorn (1954) have described what are probably the oldest known structurally preserved plants. From the pre-Cambrian Gunflint Iron formation of Ontario they report remains that include algae, possibly of cyanophycean affinities, and two forms of fungus mycelium. The fact that these fossils have been isolated from the enclosing rock with hydroflouric acid leaves little doubt as to their organic nature. The authors report that the age of these fossils "may approach two billion years". This discovery implies that the thallophytes existed for half a billion to a possible one and a half billion years before plants made their appearance on the land. It would seem to suggest two points of significance to anyone interested in the early development of the earth.

First, that plant life, as we recognize it today, became established soon after conditions became possible for its existence; in other words this suggests a rather rapid evolution from the strictly inorganic to primitive life forms such as the blue-green algae and the simpler fungi.

Second, that the development of plant life on the land succeeded only after a very long struggle extending through a billion or more years.

We have chosen to insert a few comments on *Prototaxites* because of this supposed alga's problematical relationships and the general interest that it has created since the time of its original description by Dawson in 1859. Several recent contributions dealing with *Prototaxites* have appeared in the literature. Chiarugi (1934a, b) has reported it from the Libyan desert and Arnold (1952a) has described beautifully preserved specimens from the Upper Devonian of Ontario (the genus is now known from the Upper Silurian to the Upper Devonian) and his account includes a review of the better known species. Corsin, in a comprehensive paper (1945), has described under *Prototaxites* an association of fossils from France, which he believes represent " rhizomes", leaf-like organs, and globose bodies that may have been air bladders attached to the leaves. As the " leaves ", " floats", and " rhizomes " are not connected and the anatomy of the leaves and floats is not known, his restorations must be accepted with some reservations. If they do represent the plant as it appeared in life it supports the concept of *Prototaxites* as a large marine brown alga occupying a habit comparable with that of the larger modern Phaeophyceae.

Several studies dealing with the fossil bryophytes seem worth mentioning. Walton (1925, 1928) presented evidence indicating the existence in the Upper Carboniferous of anacrogynous hepatics that compare with the modern *Metzgeria*, *Riccardia*, *Treubia*, and *Fossombronia*, and later he (1949a) described fossils that suggest that *Riccia*-like forms existed at the same time. In a discussion of bryophyte spores Mrs. Knox (1939) has suggested that many of the lesser-known Carboniferous spores may be those of bryophytes.

Harris' (1938) comprehensive account of the Rhaetic *Naiadita* suggests a liverwort resembling *Riella* of the Sphaerocarpales. More recently Lundblad (1954) has described three new genera of liverworts from the Rhaetic-Liassic of Skromberg, Sweden: *Ricciopsis*, based on thalloid remains, which may be referable to the Ricciineae; *Ricciisporites*, which is based on spore tetrads comparable with those of the living *Riccia curtisii*; and *Marchantiolites*, a thalloid hepatic with air pores similar to those of the Marchantineae. Lundblad summarizes with the comment that " The Swedish material affords conclusive evidence that plants with indubitable Marchantiales characters existed in the Older Mesozoic. This is confirmed by suggestions from other sources indicating that the major groups of Hepatics might have been differentiated as early as in the Palaeozoic." The latter comment already has considerable support from Walton's studies.

A few words may be included on the problematical Devonian fossils variously referred to *Sporocarpon*, *Foerstia*, and *Protosalvinia* in view of a recent contribution by Arnold (1954). This author has transferred to *Protosalvinia* the species formerly assigned to *Foerstia* and has described *Protosalvinia furcata* (Dawson) Arnold in some detail. The sporocarps of this species range from 2 to 5 mm in width and consist of an enlarged bilobed " branchlet " with two to eight spore tetrads borne on the inside surface. The structure of the sporocarp in places suggests hypha-like threads, and there is a concentration of waxy material at the tips. In conclusion Arnold notes that " The position of *Protosalvinia furcata* in the plant kingdom is unknown, but it had evolved to a level comparable to that of the lower bryophytes ".

Early Vascular Plants

Interpretative discussions may well play a very significant role in the development of a science. In this connection we wish to draw attention to Leclercq's recent paper (1954) " Are the Psilophytales a starting or a resulting point". We now have a very varied assemblage of fossils referred to the psilophytes. They are varied in morphology as well as the plant parts that are fossilized and the quality of preservation. *Rhynia* undoubtedly stands in the minds of most morphologists as being typical of the group, at least in its simplest form. *Psilophyton*, another fairly well-known genus, has been considered as a typical member of the group but here, as Leclercq points out,

doubt begins to creep in. *Psilophyton princeps* probably bore terminal sporangia not unlike those of *Rhynia* (Lang, 1931; Croft & Lang, 1942); *Psilophyton wyomingense* is associated with *Bucheria ovata*, the latter consisting of two rows of ovate bodies (thought to be sporangia) disposed on opposite sides near the termination of a branchlet (Dorf, 1933); *Psilophyton pubescens* bears sporangia on incurved bifurcated branches and has a vascular strand preserved in part, which consists of regular radial series of thick-walled cells mixed with ray-like bands of tissue, thus presenting a very striking contrast with *Rhynia* (Kräusel & Weyland, 1938). In the case of *Gosslingia breconensis* (Croft & Lang, 1942) the position of the sporangia on the shoot just below the point of departure of a branch is an enigma, and in this character it presents a striking contrast with *Rhynia*.

The presence of *Baragwanathia*, seemingly a perfectly valid lycopod, in the Middle Silurian and other lycopods such as *Drepanophycus* and *Protolepidodendron* in the Lower to Middle Devonian as well as the more recently described *Colpodexylon* (Banks, 1944), *Protolepidodendropsis* (Hoeg, 1942) and *Bergeria mimerensis* (Hoeg, 1942) render it difficult to assume a very close relationship with the " typical " contemporary psilophytes.

The articulates, represented by the Middle Devonian *Calamophyton* and *Hyenia*, are well differentiated; the stamp of the race is clear and it seems likely that it existed for some time prior to the Middle Devonian.

Studies dealing with the pteropsids, more specifically those members of this group that may be called ferns or fern ancestors, have been especially rewarding in recent years. The coenopterids (considered in a later section) compose a unique and rather diversified Carboniferous assemblage, and in the Devonian we now have a considerable group of fossils that, perhaps for want of a more educated guess, may be called " ferns ". *Archaeopteris latifolia* with its fern-like foliage and heterospory is of particular interest. Arnold's comment (1939, p. 311) that " it might not be amiss to venture a suggestion that the genus possibly represents one of the so-called ' missing links ' in the evolutionary sequence of the plant kingdom which are frequently postulated but seldom seen " does not seem to be unduly speculative, although much remains to be learned about the rest of the " chain ". Our knowledge of the Middle Devonian *Eospermatopteris-Aneurophyton* complex indicates a plant of forest tree dimensions and far advanced from the Rhynie plants (as representing supposedly typical psilophytes of the Middle Devonian). *Protopteridium minutum* (Halle, 1936) from the Lower Devonian of Shansi with its flattened foliage organs suggests an early fern and *Cladoxylon*, if indeed it can be fitted into any of the known major categories of classification, may perhaps also be considered a fern. It is at any rate a plant rather far removed from the psilophytes.

In summary Leclercq notes: " If we try to visualize the past history of the Pteridophytes and come to any conclusion, it appears that the Psilophytales represent a division possibly equal in importance to that of the Lycopsides, Sphenopsides, Pteropsides, running parallel with them, instead of being their converging point."

Leclercq's concepts are at least thought provoking; whether or not they are entirely correct it is becoming evident that the major groups of pteridophytes did evolve quickly, whether from the psilophytes or an ancestral stock common with the psilophytes or possibly independently from the algae. The appearance of plants on the land after perhaps a billion years of thallophytic evolution is puzzling. Why did it take so long for this landward migration to take place ? A partial explanation that seems to have escaped the attention of palaeobotanists may be found in the magnitude of tidal action that existed prior to the Middle Palaeozoic. In " The Sea Around Us " Rachel Carson points out that when the moon was half its present distance from the earth the tidal range may have exceeded several hundred feet on some shores and that in still earlier times the range of the tide would have been even more vast, flooding inland for many miles, so that " under such conditions, no living thing could

exist on the shores or pass beyond them, and, had conditions not changed, it is reasonable to suppose that life would have evolved no further than the fishes" (p. 158)[1].

The New Albany Shale Flora

The recently conducted investigations of Hoskins & Cross (1951a, b, 1952) have again directed attention to the petrifaction flora of the upper New Albany shale (of Lower Mississippian age according to these authors), which represents one of the most significant assemblages of petrified plants yet found in North America. Prior to their work this flora was studied by Read (1935, 1936a, b, 1937), by Read & Campbell (1939) and earlier by Scott & Jeffrey (1914). Although many of the plants composing this flora are still known only from very brief descriptions, it contains nearly 30 genera, including the phaeophytes (*Prototaxites* and the problematical *Foerstia*[2]), five genera of psilophytes, three genera of lycopods, a calamarian, eight genera of ferns, eight genera of seed-ferns, and three genera referred to the Cordaitales.

Among the most striking members of the flora are the polystelic forms *Cladoxylon*, *Steloxylon*, *Pietzschia*, and *Periastron*. Two species of *Steloxylon* (Fig. 1) have been described by Read & Campbell (1939); these are stems consisting of numerous vascular bundles with secondary wood, the bundles being dispersed through the stem in contrast to the ringed arrangement of the bundles in *Pietzschia* (Fig. 2). It is convenient to insert at this point a brief note on *Xenocladia medullosina* (Fig. 5) described by Arnold (1952b) from the Middle Devonian of Erie County, New York. This species is based on stem fragments with a peripheral ring of radially elongated xylem strands and oval or round strands toward the center. Arnold considers that it resembles *Pietzschia* most closely.

Although it has not been described in detail, enough is known of the genus *Siderella* Read (Fig. 4) to distinguish it as one of the most unusual and morphologically perplexing plants found in the New Albany shale flora. *Siderella* was a small plant with a hexarch or decarch, stellately lobed siphonostele. The xylem is apparently all primary, although radially seriated elements reminiscent of those found in *Schizopodium davidi* Harris commonly appear in the embayments between the xylary protuberances of the stele. Mesarch protoxylem groups are found near the extremities of the xylem "arms" and also near the internal periphery of the stele. The most unusual features of this plant, however, lie in the nature of the appendages and their mode of emission.

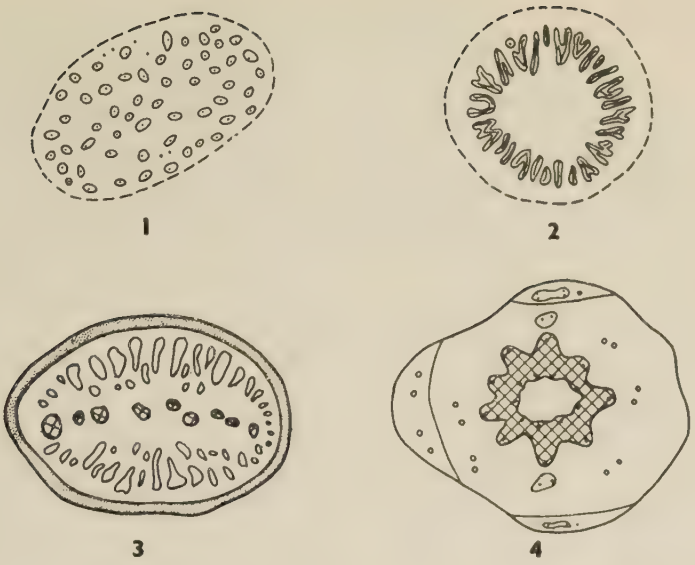
Two types of appendages are given off; the more conspicuous of these consists of opposite pairs of "phyllophore" traces, which are at first triangular in outline, but gradually assume the shape of an "H". From those arms of the stele that are not at that time producing phyllophore traces, small, concentrically constructed, simple traces are given off in whorls; these divide once or twice while passing through the cortex.

The difficulty of interpreting this plant with its whorled "phyllophoric" and "foliar" bundles was reflected in Read's reluctance to assign *Siderella* to any presently known group of fossil plants. He stated that *Siderella* combines features reminiscent of both the primitive ferns and certain sphenopsids but is probably closer to the ferns, and he subsequently established the new order Siderellales.

Asteroxylon setchelli of Read & Campbell has been transferred to the new genus *Stenokoleos* by Hoskins & Cross. *Polyxylon elegans* is another polystelic stem, which may represent an advanced member of the actinostelic psilophytes.

Certainly one of the most significant features of this flora is its contribution to our knowledge of polystelic forms presenting unique plants in themselves and possibly links between certain psilophytes and ferns (using this term in the broadest sense), which may be tracing out a hazy path to the medullosan seed ferns of the later Palaeozoic.

1. Quoted by permission, the Oxford University Press.
2. *See* reference by Arnold (1954) cited above.



FIGS. 1-4 — Diagrammatic t.s. various genera of New Albany shale plants, showing some examples of the extreme variety of stelar configurations contained in the flora (from Hoskins & Cross, 1951b, Fig. 6). Fig. 1. *Steloxylon*. Fig. 2. *Pietzschia*. Fig. 3. *Periastron*. Fig. 4. *Siderella*.

Some Articulate Fructifications

A number of structurally preserved articulate fructifications have been described in recent years, the most comprehensive single work being Hoskins & Cross' (1943) monograph of the sphenophyllalean cone genus *Bowmanites*. This paper includes a detailed review of the history of the genus, a key to the eighteen species recognized, and a description of *B. trisporangiatus*, based on a coal-ball specimen from the Des Moines series of Iowa. *B. trisporangiatus* is distinguished by the appearance of three separate sporangiophores, each with a single terminal sporangium, borne on the upper surface of each sporophyll or unit of the " sporophyllar " disc. This contribution illustrates the great morphological variation to be found in sphenophyllalean cones; another of its values lies in its demonstration of the difficulties encountered in attempting to identify compressed plant structures with structurally preserved ones, particularly when they are as complex and variable as the sphenophyllaleans.

Another new and interesting species of *Bowmanites* (*B. bifurcatus*) was described by Andrews & Mamay (1951), based on a single well-preserved specimen from an Illinois coal-ball. In this species, each unit of the sporophyllar disc is bifurcated towards the tip, and each bears a single adaxial sporangiophore that bifurcates and bears two inverted sporangia. This is a relatively simple member of the genus and one in which the spores are monolete (Hoskins & Cross, 1943, considered trilete spores to constitute a diagnostic feature of *Bowmanites*).

Several other structurally preserved fructifications with apparent sphenopsid affinity have been reported although their exact relationships are in doubt. One of these is *Sphenostrobus thompsonii* Levittan & Barghoorn (1948), based on a single coal-ball specimen from the Des Moines series of Iowa. *Sphenostrobus* is a small verticillate cone with a tetrarch protostele. From each node arises a " sporophyllar " disc consisting of 16 basally fused sporophylls. The fertile whorls each consist of 16 sessile sporangia; these, however, alternate with the sporo-

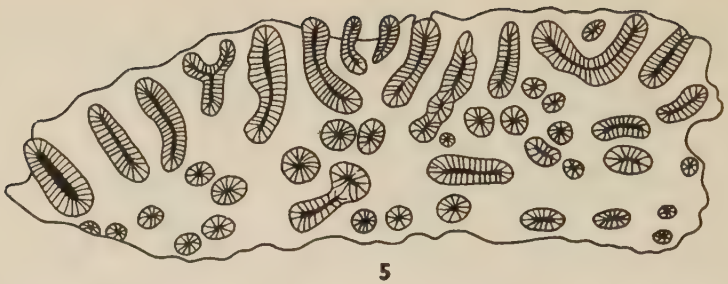


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Fig. 5 — Diagrammatic t.s. stem of *Xenocladia medullosina* Arnold, illustrating shape and disposition of the vascular bundles (from Arnold, 1952b, Fig. 1).

phylls and lie in the grooves that occur between the latter. The tetrarch stele and sessile sporangia are features that have led Levittan & Barghoorn to voice a preference for a noeggerathialean interpretation of *Sphenostrobus* rather than a sphenophyllalean or calamitalean assignment.

Mamay (1954a) has described *Litostrobus iowensis*, another articulate cone, from the same horizon in Iowa. This presents an extremely simple plan of construction; each sterile whorl consists of apparently 12 laterally fused bracts, every other one of which subtends a single, axillary, shortly pedicellate, erect sporangium; the cone is apparently homosporous. Its disc has been interpreted by the author as consisting of two alternating cycles of six bracts each; every other bract is therefore fertile, whereas the intervening ones have become sterile through reduction of parts. Certain features of the cone (sinuous epidermal cells, etc.) suggest sphenophyllalean affinities, and the author is of the opinion that, on the basis of comparisons with more complex cones from older strata, the sphenophyllalean fructifications underwent a reduction resulting in simple structures among the more advanced members.

Peltastrobus reedae Baxter (1950) and *Protocalamostachys arranensis* Walton (1949c) comprise two other problematical articulate fructifications; these are alike in that they both lack bracts or other accessory appendages interposed between the whorls of fertile ones. *Peltastrobus* consists of whorls of peltate sporangiophores, each with numerous (30 to 40) sporangia borne in two or three concentric cycles on the inner (adaxial) surfaces and margins of the peltate discs. This fructification from upper Middle Pennsylvanian coal-balls of Indiana, is interpreted as being more closely allied to the Sphenophyllales than to the Calamitales; the appearance of sinuous cell outlines in the sporangia is apparently an important feature in arriving at this conclusion. *Peltastrobus* is of further incidental interest in that its spores contain recognizable nuclei.

Although it resembles *Peltastrobus* in lacking sterile appendages, *Protocalamites arranensis* Walton, from the Lower Carboniferous of Scotland, is more simply constructed. The sporangiophores, which seem to be arranged in whorls terminate in four adaxially bent ultimate divisions, each bearing a single sporangium. Walton points out the similarity of *Protocalamostachys* to several other entities, previously known only as compressions, and particularly to *Pothocites grantoni* Paterson.

Referring to vegetative remains, we may note briefly a rather remarkable compressed specimen, *Prosseria grandis*, reported by Read (1953) from the Upper Devonian of New York State. This fragmentary specimen, apparently of articulate (pseudobornialean ?) affinity, has a thick jointed stem with greatly enlarged nodes from which orginate both leaves and branches. The latter seem to arise in opposite pairs, while the extremely long (to 33 cm) and narrow (6 mm) leaves are interpreted as originating in fascicles of three. In consideration of the large size of the leaves and the Devonian age of the specimen, Read feels that *Prosseria* may be inter-

preted as evidence that articulate leaves underwent a reduction of megaphylls rather than expansion of microphylls.

The Noeggerathiales, a group of late Palaeozoic plants possibly related to the articulates, will be considered briefly. Included in the order are *Noeggerathia, Noeggerathiostrobus, Tingia, Tingiostachya, Discinites, Saarodiscites, Saaropteris* and perhaps others. In a rather exhaustive discussion of these plants, Isabel Browne (1933) expressed the viewpoint that their relationships are probably more intimately interwoven with the Sphenophyllales than with any other group of pteridophytes; this opinion has been shared by Emberger (1944, p. 198). Certain structural features of the fructifications offer strong points in favour of such a view, although the supposedly related foliage forms are more difficult to interpret.

A somewhat different viewpoint is expressed by Hirmer (1940), who, in a paper describing certain new noeggerathialean forms, presents the following classification of the Filicales:

a)—Coenopteridineae b)—Ophioglossineae
c)—Noeggerathiineae d)—Eufilicineae

This classification strikes the present authors as a rather startling concept, in consideration of the fact that *Discinites* Feistmantel, *Saarodiscites* Hirmer, and *Tingiostachya* Kon'no present the appearance of strobiloid fructifications with the sporangia borne on the adaxial surfaces of the sporophyllar units.

In a recent discussion of this group Halle (1954) has given a tentative restoration of the " cone " of *Noeggerathiostrobus bohemicus*. It is shown as consisting of two rows of half-cup shaped scales each of which bears several sporangia on the inner (upper) surface. Halle has isolated both micro- and megasporangia from his specimens (from Rakonitz, north-west of Prague) and was able to demonstrate macerated whole megasporangia containing 16 megaspores. In conclusion, he comments as follows on the affinities of the group: " In the present case it would seem a safer course — whatever headlines be adopted for practical purposes — to regard the Noeggerathiineae as an isolated group of *pteridophyta incertae sedis.*"

The Palaeozoic Ferns

The genus *Botryopteris* is one of the better known of the coenopterid ferns, and several studies have appeared recently dealing with its problematical anatomy. Surange (1953) has reinvestigated the anatomy of *Botryopteris antiqua*, the oldest known species from the Lower Carboniferous of Scotland. *B. antiqua* proves to be a much more complex plant than was suspected by earlier workers, presenting two morphologically distinct types of main axes or " stems ". A dorsiventrally constructed organ has been shown to give rise to a radially symmetrical stem from which roots and petioles depart in a spiral arrangement. The petioles branched sympodially, producing complex nonlaminate " fronds ". The radial stems branched dichotomously and the young fronds are known to have been circinately coiled, lending strength to the filicinean interpretation of these primitive plants. The dorsiventral axes apparently produced only the radial stems. Surange also notes that a similar dorsiventral axis was present in the Upper Carboniferous *B. ramosa.*

A similar type of organization was described earlier by Long (1943) for *B. hirsuta*, based on English coal-ball specimens. In this example a dorsiventral structure with three adaxial protoxylem teeth (main rachis) gives rise to a circular stele (bud), which in turn gives rise to petiole traces and roots. Although the overall pattern is comparable with that described for *B. antiqua*, Long & Surange disagree on morphological interpretations. It is Surange's belief that Long's " main rachises " are morphologically equivalent to the " dorsiventral axes " of *B. antiqua*, and that Long's " buds " are the same as the " radial stems " of *B. antiqua.*

A third species has been described, *B. trisecta*, from the Pennsylvanian of Illinois by Mamay & Andrews (1950) in which the protostelic radially symmetrical stem bears " fronds," which are three dimensional. The initial foliage strand produces two primary pinna traces in

quick succession; these in turn trisect at a plane at right angles to the previous one, and in each case one of the secondary pinna traces is protostelic, resembling very closely the stem stele rather than the W-shaped rachis strand. *B. trisecta* thus is of interest in that its appendages are distinct units arranged on the stem after the fashion of fronds, but the latter are three dimensional and in so far as presently known, without a lamina, thus presenting characters of a leaf, yet one that is but little advanced from a unit of the shoot system.

In *B. trisecta* it was not possible to follow the main rachis strand to determine whatever branching pattern it underwent. Delevoryas & Morgan (1954) have described specimens from the same locality in which a W-shaped strand (possibly equivalent to the main rachis strand of *B. trisecta*) produces pinna traces of several orders, the smallest of which bear lobed pinnules 1·5 cm or more long.

Several studies have appeared dealing with the fertile parts of ferns, which provide us with a broader insight into the morphological and evolutionary relationships of certain fern groups and which strengthen the opinion that at least three modern families (Marattiaceae, Gleicheniaceae, and Schizaeaceae) had their roots well established in the Palaeozoic. Significants trides have also been made with the coenopterids. Three species of *Botryopteris* have been described based on fructifications (Mamay, 1950). The primary interest of this report lies in the fact that although the sporangia themselves are very difficult to distinguish, their contained spores, upon which the differentiation of species was primarily based, exhibit such a wide range of morphological differences that some doubt is cast upon the feasibility of attempting to determine the natural affinities of some groups of " sporae dispersae ". It is of further interest to note that the structure of these sporangia suggests that true leptosporangy may have been closely approached by the coenopterids.

The fructifications of *Stauropteris burntislandica*, another plant of coenopterid affinity, from the Pettycur limestone (Lower Carboniferous) of Scotland, have been investigated by Surange (1952), with rather startling results. Surange's investigation demonstrates that the spindle-shaped structures, previously interpreted as " glands " of this species and named *Bensonites fusiformis* by Scott (1908), are actually megasporangia, each containing two megaspores. These structures were terminally borne on the ultimate branchlets of the complex, nonlaminar " fronds " of the plant. This discovery of the heterosporous habit among plants of otherwise primitive aspect leaves one wondering just how complex a group the coenopterids will ultimately prove to be.

Several apparent marattiaceous fructifications have also been described by Mamay (1950) from Pennsylvanian coalballs of the United States. These include various species of *Scolecopteris* and *Cyathotrachus*, as well as the new genus *Eoangiopteris*, characterized by its linear synangia, which present a close resemblance to the sori of the modern marattiaceous genus *Angiopteris*. Evolutionary relationships are discussed, and the possibility is suggested that marattiaceous fructifications may have arisen by a " phyletic slide " of the sori, from coenopterid ancestral stock of the *Chorionopteris* type in which asterothecoid synangia are borne marginally on the lamina. It is also shown that, at least in the genus *Scolecopteris*, spore morphology cannot be considered a dependable diagnostic feature at the generic level, for both trilete and monolete spores are found in this genus.

Applying acid maceration techniques to compressed specimens from the Carboniferous and Permian of central Europe, Remy (1953) has described the structural details of several unique fructifications, some of which are probably pteridospermous (discussed on a later page), whereas others are of marattiaceous or other filicinean relationships. One of the most interesting and problematical is the genus *Saarotheca*. Borne on sphenopteroid foliage, the fructifications (*S. sphenopteroides*) are of very complex organization, apparently consisting of an elongated central receptacle, to which are attached 20 to 25 spheroidal sporangia containing small bilateral spores; the entire structure is surrounded by a massive-walled indusium-

like envelope, the dehiscence mechanism of which is not known (Fig. 6). Remy avoids speculation regarding familial affinities of this peculiar plant, merely indicating a high probability that it is a true fern. Remy's reconstructions do suggest hymenophyllaceous or cyatheaceous sori, and it may also be noted that an affinity with the water ferns does not seem to be beyond the realm of possibility.

Abbott (1954) has presented an interesting and useful study of the American Pennsylvanian specimens of *Oligocarpia*, a genus whose fructifications indicate true gleicheniaceous affinity. In this revision of the genus nine species have been recognized, based primarily on numbers and arrangement of the sporangia in the sorus. This study was aided by the application of an effective transfer method for removing large specimens of compressed plant fossils from slabs of shale (Abbott, 1950; Abbott & Abbott, 1952). Another significant technique development that deserves mention is the use of plastic embedding material for studying stelar cellular structure of compressions; developed by Leclercq & Discry (1950), the great potentiality of this method has been demonstrated by Leclercq (1951) in her investigation of *Rhacophyton*.

The Pteridosperms

The problem of the limitations of this taxonomic group is essentially the same as "what is a psilophyte?" or "what is a fern?" and, as might be expected, the more we learn about the group the more indefinite the boundaries become. We are, therefore, including in the discussion below a considerable assemblage of fossils all of which seem to fit this category better than any other.

Several distinctive microsporangiate organs have been described. Schopf (1948) has given a comprehensive account of *Dolerotheca*, there being strong evidence to indicate that this is the microsporangiate organ of the Medullosas, and Baxter (1949) has described additional species. Two other possibly related fossils seem to deserve special note: *Paracalathiops stachei* described by Remy (1953) from the Carboniferous of Germany and *Lacoea seriata* described by Read (1946) from the Pennsylvanian of Pennsylvania. In both cases these consist of a cluster of elongate sporangia enclosed by sterile

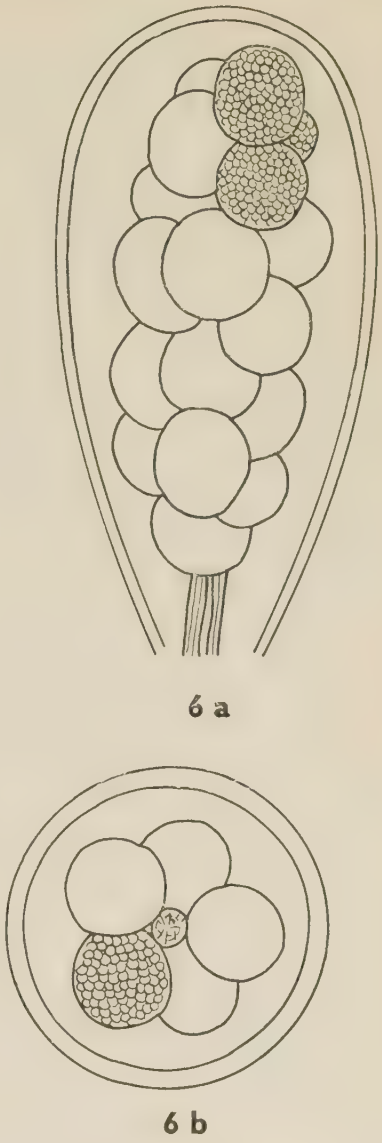


Fig. 6 — Reconstructions showing the longitudinal (*a*) and transverse (*b*) aspects of *Saarotheca sphenopteroides* Remy, with its centrally located receptacle, numerous sporangia, and surrounding "indusium" (from Remy, 1953, Fig. 2).

cupulate lobes. Prior to the discovery of these fossils the only cupulate microsporangiate organ of supposed pteridospermous affinities was *Potoniea* (Halle, 1933). It may be noted in passing that Halle (1942) has also described a rather unique species of *Potoniea* (*P. strigulosa*) in which the "cupule" and tips of the sporangia are covered with bristle-like hairs.

One cannot help speculating on the possible homology of these fructifications with multiovular cupulate organs such as *Gnetopsis elliptica* and *Calathospermum scoticum*. The latter was described briefly by Walton in 1940 and more fully in 1949b. *Calathospermum* is a large six-lobed cupule, about 45 mm long, from the Lower Carboniferous of Dunbartonshire, which encloses several dozens of seeds. The latter appear to be highly specialized and compare quite closely with the seeds described as *Salpingostoma dasu* by Gordon (1941).

The evidence indicates that by no means all of the pteridosperms bore their seeds enclosed in cupules; those that did are, of course, of special interest because of the possibility of angiosperm ancestry. There is no reason to suppose that the cupulate microsporangiate organs noted above were borne on the same plants that bore cupulate seeds, yet the suggestion of homology is tempting. The concept of the origin of the integument as an enclosing ring of telomes (which may have been sterilized fertile ones or originally sterile) is well known and need not be considered in detail here. With *Calathospermum* and *Paracalathiops* we are dealing with clusters of seeds and sporangia respectively, which are enclosed in cupulate lobes, there being no reason to suppose that these were anything other than originally sterile telomes. It is clearly evident that in either case we are dealing with large numbers of fertile structures enclosed by a ring of sterile ones. It may be that the seed-bearing organs (such as *Calathospermum*) resulted from a secondary enclosure of certain sporangia (integumentation of a single sporangium) in which only one spore matured and thus assumed the characteristics of an ovule.

Walton (1949d) has described another fructification from the Kilpatrick Hills of Dunbartonshire, which deserves at least brief mention here. In two nodules, fragments of well-preserved fructifications were found which, on the basis of resemblances between the exposed surfaces of the specimens and compressed fructifications previously described under the name *Alcicornopteris*, Walton has referred to a new species of that genus (*A. hallei*). Thin sections revealed the fructifications to consist of a system of many small, branching rachides, from which project small clusters of pedicels, which in turn bear sporangia. The sporangia are slender, exannulate structures that apparently bore only one kind of spore. On the basis of the exannulate sporangia, position of the thickening of the sporangial wall cells, sclerotic nests in the rachides, and structure of the vascular strands, Walton refers this fructification to the pteridosperms. He also speculates on the possibility that *Alcicornopteris* may be a form intermediate between the fruiting habit of some of the Upper Devonian archaeopterids and the Upper Carboniferous synangial fructifications. It is suggested that *Alcicornopteris* may be the precursor of some of the synangial pteridospermous fructifications such as *Potoniea* or *Dolerotheca* but no relationship is suggested with the *Whittleseya* type of synangium.

A recent discovery reported by Jongmans (1954) adds a very significant note of confidence to our use of the term "pteridosperm". In a collection of plants from the lower part of the Westphalian "A" in Holland he found a remarkable series of specimens that definitely establish the relationships between male, female and foliar parts. The foliage, *Neuropteris schlehani*, was found in organic connection with both *Whittleseya media* Stockmans and *Pachytesta*-like seeds. The author points out that the mature seeds are found only as isolated specimens while those that occur in organic connection with the leaves appear to be immature stages. Since so much of our knowledge of the pteridosperms is based on fragmentary or isolated parts the significance of this contribution is obvious.

A considerable number and variety of pteridospermous stems have been described in the past decade, most conspicuous

being the genus *Medullosa*. The stems assigned to *Medullosa* vary greatly in size and in the number of units composing the polystelic vascular system. In the simpler forms such as *M. distelica* Schopf (1939) and *M. pandurata* Stewart (1951) two steles are present whereas in *M. grandis* Andrews & Mamay (1953) there are 20 or more; the latter is also the largest Carboniferous species known, the stelar system attaining transverse dimensions of 12×28 cm and presenting a striking contrast to *M. endocentrica* in which it does not exceed 1·2 cm in diameter. Detailed reviews of recent vintage of this complex genus have been given by several authors and need not be reported here; for more exhaustive accounts the reader is referred to the contributions of Andrews & Mamay, Baxter, Delevoryas, Roberts & Barghoorn, Schopf, Steidtmann, and Stewart cited in the bibliography.

A few remarks concerning a particularly interesting species, *M. heterostelica* (Stewart & Delevoryas, 1952) may, however, be of general interest. This species comes from the Pennsylvanian of Kansas and is a relatively small one displaying two steles in the internodal region. These two steles divide to form as many as 23 steles at the node; some depart as foliar strands whereas the others reunite slightly above the node to reform the bistelar internodal organization of the stem.

Numerous Carboniferous pteridospermous seeds have been described, several of which are of particular interest. Long (1944) has described a specimen of *Lagenostoma ovoides* containing a beautifully preserved gametophyte bearing three archegonia with egg cells. The gametophyte has also been described for *Cardiocarpus spinatus* (this probably being a cordaitean seed) in which the archegonia are recognizable (Andrews & Felix, 1952). Of the larger Pennsylvanian seeds Hoskins & Cross (1946) have given a comprehensive account of *Pachytesta* and more recently Stewart (1954) has given an excellent account of *P. illinoense*. Mamay (1954b) has reported a well preserved cupulate seed under the new binomial *Tyliosperma orbiculatum*; the cupule is a delicate structure, deeply and irregularly lobed and bearing numerous nonglandular multicellular emergences. In concluding these brief comments on seeds it is regrettable to note that Palaeozoic seeds with embryos still remain on the " wanted " list !

The affinities of the Permian leaves known since the early part of the last century as *Glossopteris* have long stood as one of palaeobotany's great enigmas. In a particularly significant contribution Plumstead (1952) has described a considerable

FIGS. 7-10 — Fructifications. of *Glossopteris* (from Plumstead, 1952, Figs 1b; 1a; pl. 44, Fig. 3; and Fig. 2, respectively). Fig. 7. " Open cupule " of *Scutum leslium*, borne on leaf of *Glossopteris browniana*. Fig. 8. " Closed cupule " of *Scutum leslium*. Fig. 9. *Scutum leslium*, " Ripe, fertile half of an open cupule, showing burst sacs with crater-like hollows from which he seeds have fallen." Fig. 10. " Open cupule " of *Scutum dutoitides*, attached to leaf of *Glossopteris indica*.

collection of specimens from the Lower Permian rocks of Vereeniging in the Transvaal, South Africa, which bear reproductive organs thought to be seed-bearing cupules. Five species are described under the new generic name *Scutum*. This apparent reproductive organ is described as being composed of a round, lanceolate or ovate bilaterally symmetrical cupule attached by a pedicel to the midrib of the *Glossopteris* leaf (Figs. 7-10). What is referred to as the fertile half of an open cupule bears a central head in which are embedded a " number of small oval sacs ranging from 1 to 2 mm in size ", and the entire head is surrounded by a wing.

A second genus (*Lanceolatus*) of reproductive organs is also described, the morphology of which is not as clearly defined.

This is a discovery of great importance, but much remains in doubt concerning the morphology and affinities of the organs. It seems clear that they are reproductive structures and equally clear that they are attached to *Glossopteris* leaves. The head with its embedded bodies bears some resemblance to certain bennettitalean seed fructifications. However, it is not proved beyond doubt that these are seeds.

Plumstead's paper is appended with comments from several palaeobotanists. Professor Walton offers a suggestion that to us seems worth considering — that the fructification is not a cupulate head but rather a strobilus, the supposed cupule actually consisting of laminate extensions of the closely aggregated seeds (or sporangia ?).

Assuming, however, that the organ is cupulate Plumstead offers evidence to suggest that the cupule was closed after fertilization, a fact, if it is such, that allows the possibility of angiosperm relationships. The affinities are assumed by the author to be with the pteridosperms, although, as several of the critics point out, this conclusion can be accepted only as a tentative one.

Another interesting and distinctive group of fossils, which also suggest pteridospermous affinities, have been described on the other side of the Atlantic. Under the name of *Zuberia zuberi* Frenguelli (1944) has reported a unique association of fossils from the Triassic of Argentina. The dichotomously forking sterile frond bears pinnules of the *Odontopteris* type (Fig. 11). The microsporangiate organ (Fig. 12) is a dichotomously forking branch bearing numerous appendages that consist of a pedicel beyond which many sporangia are attached at right angles to the appendage axis. Associated with the sterile fronds are cupulate organs (Fig. 13), the preservation of which is apparently rather poor.

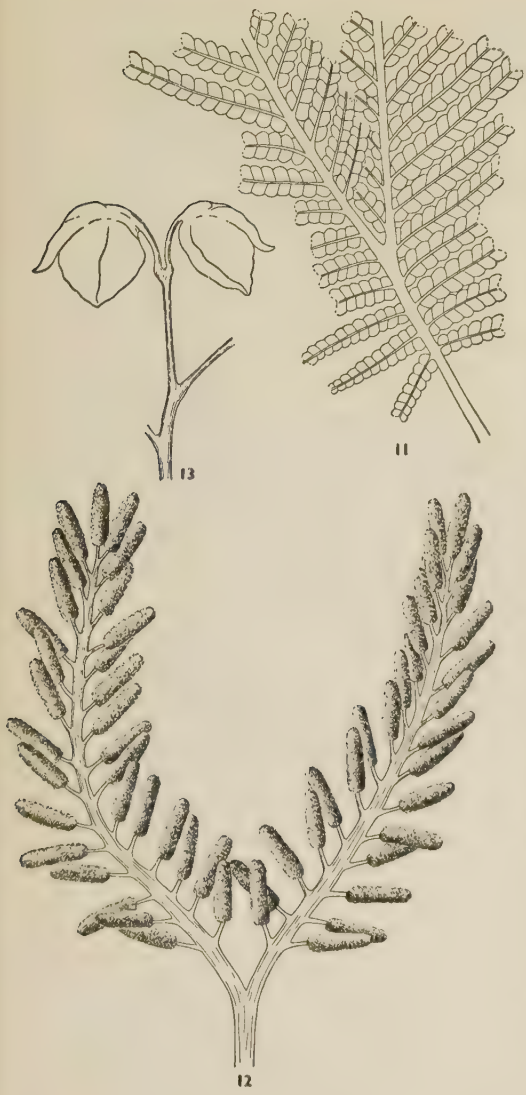
There is a rather striking similarity between this assemblage of fossils and the Corystospermaceae, which Thomas (1933) described from the Triassic of Natal. The cupulate organs *Umkomasia* and *Pilophorosperma* show a resemblance to the cupulate organ of *Zuberia*, and the fertile heads of the (microsporangiate) *Pteruchus africanus* are comparable to the corresponding structures of *Zuberia*. With reference to the foliage, the *Dicroidium* sp. figured by Thomas (1933, Fig. 50) is strikingly similar to *Zuberia zuberi* figured by Frenguelli on plate 4.

Although much remains to be known about this assemblage of fossils it appears to be pteridospermous in the broad sense and adds a significant chapter to the Mesozoic members of this group.

One other extension of the pteridosperm record may be mentioned briefly: Brik (1941) has reported male flowers of *Caytonia* (the new species *C. palibinii*) associated with *Sagenopteris* foliage " In stratis liassicas loci Taschuten dicti in Asia Media ".

Cordaites and Conifers

Perhaps the most complete evolutionary sequence that has ever been worked out for the plant kingdom is Florin's study of the female reproductive organs in the cordaites and conifers. The original account appeared in eight monumental parts in volume 85 of *Palaeontographica*. Florin has given us a condensed account in English in " Evolution in Cordaites and Conifers " (given as the John M. Prather Lectures at Harvard University in 1948-49; Florin, 1951), and as this has been rather widely distributed we include here but a brief summary. In addition to the account of the evolution of the female



FIGS. 11-13 — Fig. 11. Frond of *Zuberia zuberi* (Szajnocha) Frenguelli (from Frenguelli, 1944, pl. 4). Fig. 12. Reconstruction of the male fructification (*Pterorrachis barrealensis*) of *Zuberia zuberi* (from Frenguelli, 1944, Fig. 12). Fig. 13. Cupulate fructification of *Zuberia zuberi* (from Frenguelli, 1944, Fig. 10).

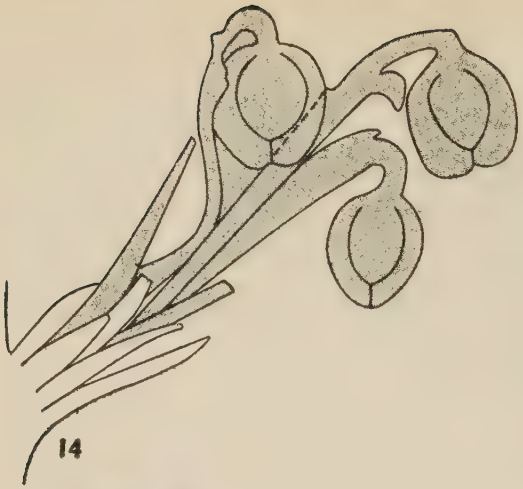
reproductive organs in the cordaites and conifers this includes a consideration of the origin of the integument of cordaitean seeds, the female reproductive organ of the taxads and the application of the telome theory to the reproductive organs of the cordaites, conifers, and taxads.

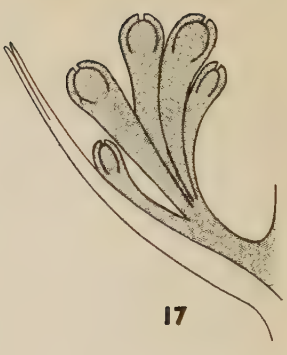
Two main types of female inflorescences are described for the Carboniferous cordaites: *Cordaianthus pseudofluitans* in which the megasporophylls are branched and bear more than one seed (Fig. 14); and a somewhat later reduced type represented by *Cordaianthus zeilleri* in which the megasporophylls were unbranched and uniovulate.

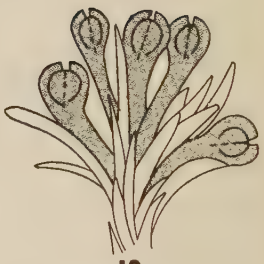
In the Upper Carboniferous *Lebachia piniformis* (Fig. 15), the affinities of which appear to lie closer to the conifers than cordaites, the dwarf shoots are aggregated into distinct cones with one megasporophyll in each shoot bearing a single seed. In *L. goeppertiana* (Fig. 16) numerous appendages compose the dwarf shoot and a single fertile one is present, which occupies a position on the upper (adaxial) side of the shoot.

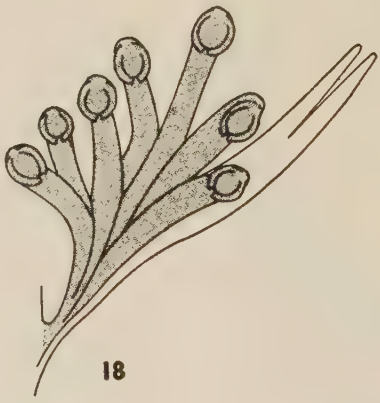
A considerable degree of variation is evident in the lower Permian members. In *Ernestiodendron* the dwarf shoots are more or less flattened, bearing relatively few sterile appendages or none at all as in *E. filiciforme*, the dwarf shoot of this species consisting of three to five sporophylls (Fig. 17). In *Walchia* (*Ernestiodendron*) *germanica* (Fig. 18) " sterile scales have disappeared almost completely; only one such scale is sometimes found at the base, while there are three to seven megasporophylls with inverted ovules". *Walchiostrobus* (*Ernestiodendron* ?) species are described in one of which the sterile appendages are numerous with erect ovules borne on the fertile appendages (Fig. 19), whereas in another the ovules are inverted (Fig. 20).

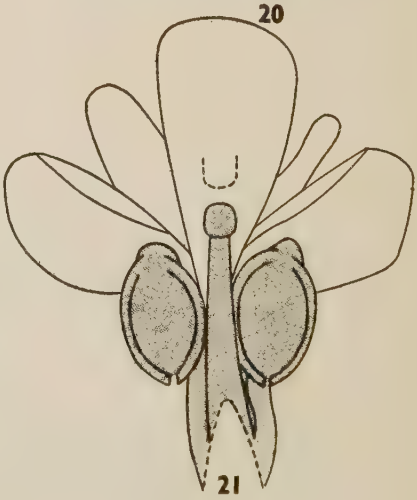
In the upper Permian *Pseudovoltzia liebeana* (Fig. 21) the dwarf shoot consists of five flattened sterile scales of unequal size with three fertile appendages borne on the upper surface, each bearing a single inverted ovule. In *Ullmannia bronii* the dwarf shoot consists of but a single sterile scale with a single fertile appendage, bearing an inverted ovule on its upper surface. Seemingly more primitive forms such as *Voltziopsis africana*, with several sterile and fertile scales, bearing erect ovules, lingered on into the Lower Triassic.

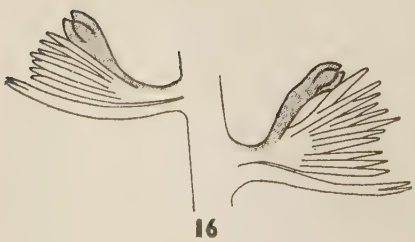












FIGS. 14-21.

It seems clear that Florin's studies have clarified the much speculated morphology of the ovuliferous scale and bract as it is known in such modern conifers as *Pinus*; it is particularly interesting also to find that the evolution of this structure was very nearly completed by late Palaeozoic time. The modern conifers stand as a remarkable group of plants in the relatively little evolution that they have undergone in the past 150 million years, yet they have competed with considerable success against all other groups during that long span of time.

Florin (1952) has also written an excellent summary of the *Metasequoia* story, which includes a comprehensive bibliography of that genus, living and fossil.

It seems appropriate to mention at least briefly Dr. Calder's recent work on the rather well known Patagonian (Cerro Alto) petrified forest of probable Mesozoic age, which is perhaps best known from Wieland's (1935) account. Calder (1953) offers evidence to indicate that only two species are represented, *Araucaria mirabilis*, which belongs to the *Bunva* section of *Araucaria*, and *Pararaucaria patagonica*, the affinities of which probably lie with the Taxodiaceae. Of particular note is her description of seedlings of *A. mirabilis*; these occur rather abundantly and seem to have been correctly interpreted for the first time by Calder.

The Ginkgophytes

Florin (1949) has redescribed the problematical *Trichopitys heteromorpha* and presented a rather strong case in favour of its ginkgophyte affinities. Two specimens from the upper Permian are described, which consist of long shoots bearing finely dissected leaves with " fertile complexes " in the axils. The leaves, or vegetative telome systems, attain a length of 10 cm and appear to be more strongly developed on one (the upper) side of the shoot than the other; the fertile complexes are also, for the most part, confined to this side of the long shoot.

In one specimen the axillary fertile complex bears up to six terminal, inverted ovules. In the other specimen described as many as 20 ovules are borne by a single complex. It is believed that these fossils represent a part of the plant homologous with the long shoot of the modern *Ginkgo*, the short-shoot habit, with attendant segregation of the reproductive structures, having been evolved quite early in the Mesozoic. Florin (1936) has in fact demonstrated the existence of the short-shoot habit in ginkgophytes in his consideration of the Mesozoic fossils from Franz-Joseph-Land. Kräusel (1943) has also described an interesting assemblage of ginkgophytes from the Triassic of Lunz, his descriptions being accompanied by excellent restorations.

In his *Trichopitys* paper Florin (1949, p. 102) gives a useful list of the fossils that are considered to be of ginkgophyte affinities. This consists of 17 genera based on foliar remains ranging from the lower Permian to the present. Quoting from his summary remarks (p. 101) he notes " *Trichopitys* has no leaf-bearing short shoots of the types found in several Mesozoic and Caenozoic Ginkgophytes, but from recent morphological studies carried out by Gunckel and Wetmore and others it is evident, as might be expected on theoretical grounds, that the development of the short shoot habit is a secondary feature in *Ginkgo* and that its ancestors were once characterized by exclusively exhibiting the long-shoot habit. *Trichopitys* is no ancestral link with the primitive Conifers, nor is it closely related to the Cordaitales. The

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Figs. 14-21 — Diagrammatic reconstructions of cordaitean and coniferous ovulate flowers, with ovules and megasporophylls shaded. All reproduced from Florin (1951) as follows: Fig. 14. *Cordaianthus pseudofluitans* Kidston (Florin, Fig. 16a). Fig. 15. Posterior view of uniovulate flower of *Lebachia piniformis* (Schloth. pars) Florin (Florin, Fig. 32a). Fig. 16. *Lebachia goepperti ana* Florin (Florin, Fig. 33a). Fig. 17. *Ernestiodendron filiciforme* (Schloth. pars) Florin (Florin, Fig. 34a). Fig. 18. *Walchia* (*Ernestiodendron ?*) *germanica* Florin (Florin, Fig. 34d). Fig. 19. *Walchiostrobus* (*Ernestiodendron ?*) sp.; form with erect ovules (*Florin*, Fig. 34e). Fig. 20. *Walchiostrobus* (*Ernestiodendron ?*) sp.; form with inverted ovules (Florin, Fig. 34f). Fig. 21. *Pseudovoltzia liebeana* (H. B. Gein.) Florin; adaxial view (Florin, Fig. 36a).

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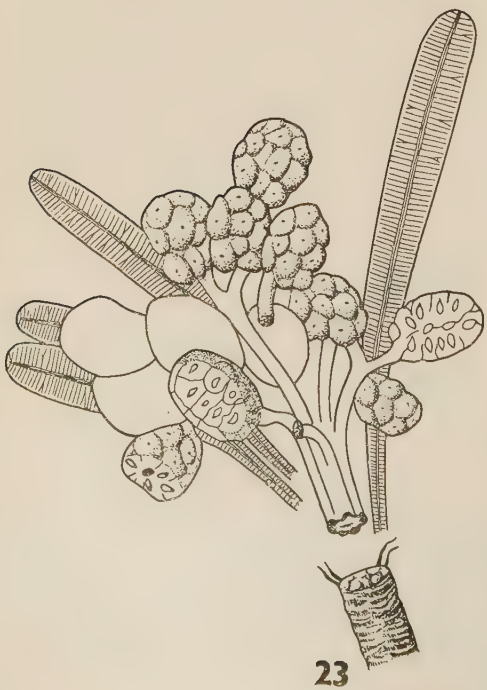

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FIGS. 22-24.

Ginkgoinae, Cordaitinae, Coniferae, and *Taxinae* undoubtedly belong to the same natural plant group of higher order, the . . . *Coniferophyta* . . ., but they constitute parallel evolutionary lines which probably were already separated from each other in Upper Devonian or Lower Carboniferous times. At all events, a clear differentiation can be seen as far back as the available fossil records go."

Some Fossils of Uncertain Affinities

Perhaps the most unique assemblage of fossil plants to be reported in recent years are the Indian Jurassic remains referred by Sahni (1948) to the new group of gymnosperms, the Pentoxyleae, the group having been studied in detail by Sahni, Rao, and Srivastava. More recently Vishnu-Mittre (1953) has described what are believed to be the male organs.

In his comprehensive summary of the work on this group Sahni (1948) notes " Some discoveries in science help, or appear to help, in the solution of old standing problems; others — and these are perhaps the more interesting — seem to create new difficulties in our path. My object here is to draw attention to a recently recognized group of plants which defies classification and presents a new problem in our understanding of the evolution of gymnosperms."

The Pentoxyleae consist of associated leaves, stems, and male and female cones and come from a Jurassic horizon in the Rajmahal Hills in India. The stems (*Pentoxylon sahnii*) reach several centimeters in diameter and consist of a ring of five, or occasionally six, closely aggregated steles. Each of the latter is composed of a tangentially elongated mesarch primary strand enclosed in secondary wood which is strongly endocentric in its development, includes uniseriate rays, has well marked growth rings, and in general is typically coniferous. Alternating with these steles are five much smaller ones consisting chiefly of secondary wood; their origin and purpose are not known. Another genus of stems, *Nipanioxylon,* has been described which consists of more numerous bundles in which the endocentric growth is less pronounced, but these are not nearly as well known as *Pentoxylon.*

The stems bore shoots 5 to 7·5 mm thick covered with an armor of closely aggregated leaf cushions (Fig. 22). It is believed that leaves, described as *Nipaniophyllum raoi* were borne on these shoots. The leaves attained a length of 7 cm, are somewhat less than 1 cm broad, and are of the *Taeniopteris* type. The vascular bundles are similar to those of modern cycads whereas the stomata are described as being of the bennettitalean type.

The female infructescence (*Carnoconites compactum* and *C. laxum*) consists of a peduncle that divided into several branches or pedicels each of which bore a seed-bearing cone (Fig. 23). Each cone in turn bore several closely compacted seeds with a thick, fleshy integument and micropyle pointing out. No interseminal scales are known. It seems very likely that these peduncles were borne terminally on the shoots.

The male flowers (*Sahnia nipaniensis* Vishnu-Mittre, 1953) were terminally borne on shoots resembling those of *Pentoxylon sahnii* and consist of a ring of filiform spirally branched appendages fused basally to form a disc (Fig. 24). Unilocular sporangia terminate short branchlets of the appendages or " microsporophylls." The pollen grains are described as " monocolpate and boat-shaped."

In summing up the affinities of these plants Sahni notes (1948, p. 79): " When we try to ascertain the place of the *Pentoxyleae* in this broad classification, we are faced with a real difficulty. While in their seed attachment they are clearly Stachyosperms (coniferophytes) and they also have a coniferous type of wood, their stomatal structure is fundamentally

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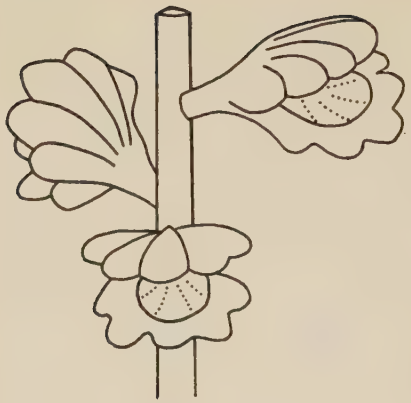
FIGS. 22-24 — Fig. 22. Reconstruction of vegetative shoots and leaves of *Pentoxylon sahnii* (from Sahni, 1948, Fig. 45). Fig. 23. Reconstruction of *Pentoxylon sahnii,* showing female cones (from Sahni, 1948, Fig. 46). Fig. 24. Reconstruction of male flowers of *Sahnia nipaniensis* Vishnu-Mittre (from Vishnu-Mittre, 1953, Text-fig. 11).



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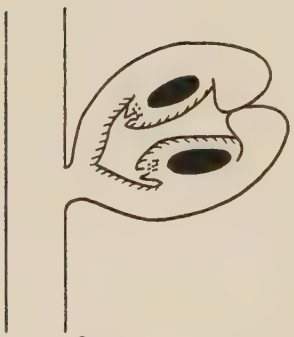

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FIGS. 25, 26 — Fig. 25. Reconstruction of part of a cone of *Leptostrobus longus* Harris (from Harris, 1951, Fig. 4D). Fig. 26. L.s. *Leptostrobus* capsule (from Harris, 1951, Fig. 4C).

bennettitalean, the vascular anatomy of the leaves is truly cycadean, and the general anatomy of the stem is unique."

Vishnu-Mittre has pointed out the general Bennettitalean plan of the male flower but notes that the microsporophylls are erect from the beginning and that the pollen is cycadean. There is little wonder that Sahni found it necessary to create the new name Pentoxyleae to include this remarkable assemblage of fossils.

The efforts of Harris in investigating the Jurassic floras of Yorkshire, England, have recently produced an extremely interesting account of the fructifications of *Czekanowskia*, and the related genera *Solenites* and *Hartzia*. *Czekanowskia* is a relatively common genus of Mesozoic leaves that has, for several reasons, consistently been classified among the Ginkgoales. Its supposed fructification, *Leptostrobus*, is a most curiously constructed, lax, cone-like structure, bearing fertile units in a distal region and small scale leaves in the basal portion. The spirally arranged fertile appendages each consist of a two-valved, somewhat flattened capsule. Each valve is five-lobed on its distal margin and contains a single seed opposite each lobe. The general morphology of this unusual cone is shown in Figs. 25 and 26.

Although *Czekanowskia* and *Leptostrobus* have never been found in organic connection with each other, Harris presents some rather strong evidence (constant association of parts and close agreement in structure of the basal leaves and cuticles) for attributing *Leptostrobus longus* to *Czekanowskia hartzi* and *L. cancer* to *Solenites vimineus*.

In spite of the evidence of relationship presented in this paper, the problem of assigning these fossils to a known group of plants becomes no easier. Harris points out that a morphological comparison of one valve of a *Leptostrobus* cone unit may be made with a single *Caytonia* fruit, a *Cycas* megasporophyll, a *Cupressus* cone scale, a several-seeded cupule of a Lower Carboniferous pteridosperm, or a two-seeded cupule of one of the Triassic pteridosperms (*Umkomasia*); however, the fact that *two* halves are involved in each unit greatly complicates the matter, and it is concluded that this complex cannot be reasonably assigned to any presently known family of gymnosperms.

In concluding this review we would like to mention a recent contribution that presents an interesting example of one of the ways in which research projects can become diverted into completely unexpected channels. During the course of investigations of coal-ball plant fossils it was found (Mamay & Yochelson, 1953) that certain coal-balls contained extremely diverse assemblages of marine animal remains; these have since been found to include foraminifera, worm tubes and pellets, conodonts, scolecodonts, corals, bryozoans, brachiopods, gastropods, cepha-

lopods, pelecypods, trilobites, ostracods, and fish fragments (shark and palaeoniscoid). In some coal-balls these animal remains are scattered throughout the matrix among the plant debris, whereas in others they are concentrated in spheroidal or cylindroidal masses of marine sediment, more or less centrally located inside an otherwise " normal " coal-ball.

Animal remains are extremely rare in coal seams, and it is difficult to explain these rich concentrations of marine animal fossils. It is assumed that some transportation of material was involved, but definite conclusions regarding means and distances involved have not been derived as yet.

Acknowledgement

The authors wish to express their appreciation to Mrs. Elinor Stromberg for the preparation of the illustrations.

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REVIEWS

HILL, R. & WHITTINGHAM, C. P. 1955. "Photosynthesis." Methuen's Monographs on Biochemical Subjects. Pp. 165. Methuen & Co. Ltd., London. John Wiley & Sons, Inc., New York. 8*s*. 6*d*.

THE literature on photosynthesis often appears as a bewildering network of studies in physics, chemistry, botany and physiology. There has been a great need for a less voluminous introduction to photosynthesis research than the unabridged works of Rabinowitch.

Hill & Whittingham have approached the problem for the biochemist and physiologist with their discussion of current trends in photosynthesis in the light of the pertinent older information. They have successfully endeavoured to give the reader a background for evaluating current work. From a brief historical section to reports on current unpublished research they have assembled a very useful and interesting discussion which engenders new ideas as one reads.

The structure and physiology of the chloroplast is followed by the chemical properties and functions of its pigments. The physiology of photosynthesis — rates of gas exchange as functions of CO_2 pressure, light intensity, inhibitors and rate of energy absorption — is reduced to the observations pertinent to current points of view. A very limited fraction of the volume is devoted to points of view which have become out of date, and these are included to present the type of reasoning which has led to current understanding. Comparative biochemistry, exemplified by the views of Van Niel, and a survey of the Hill reaction lead to the conclusion that the photolysis of water is independent of the reduction of carbon dioxide.

A detailed survey of biochemical carboxylations appears to reemphasize the importance of the carboxylation of ribulose diphosphate, the universal photosynthetic carboxylation pathway. The book was written before we studied this enzyme reaction and was brought up to date by personal communication.

The "chloroplast reaction" (Hill reaction) is elegantly discussed. The illuminated chloroplast is capable of reducing the triphosphopyridine nucleotide required in CO_2 reduction. How this is done and how oxygen is produced is not known. The authors discuss some of the current theories of quantum conversion and mechanism of oxygen production. These include a valuable summary of the possible role of the chloroplast cytochrome (cytochrome f).

The reader often wishes that the authors had taken this opportunity to present more vigorously their personal viewpoints on the many facets of this problem with which they are eminently familiar. The book is, nonetheless, exceedingly stimulating as well as enjoyable.

A. A. BENSON

INGRAM, M. 1955. "An Introduction to the Biology of Yeasts." Pp. 273. Sir Isaac Pitman & Sons, Ltd., London. 25*s*.

ALTHOUGH yeast is perhaps the first plant to have been domesticated by man, yeast cells were actually seen by him only in 1680. It took another 150 years to know that they reproduce by budding. Pasteur (1860) thought that there could be no fermentation without living yeasts but Buchner (1897) showed that an extract of ground yeast cells could bring about the same reaction. This property was destroyed by heating and was concluded to be due to the enzyme zymase.

These discoveries led the way to an intensive study of yeasts from the morphological, cytological, genetical and biochemical standpoints. The first chapter of the book deals with the general morphology and cytology of yeasts, the occurrence of haploid and diploid phases and

the possibility of producing polyploid strains by treatment of the diploids with camphor or colchicine. The next chapter is concerned with the chemical composition of yeast cells, first their organic and then their inorganic constituents. Spectroscopic analyses have consistently shown the presence of Cu, Mn, Sn and B in relatively large amounts, and Thallium, also detected, is believed to stimulate the growth of yeasts. The most important chapter, dealing with fermentation, now follows. From the mechanism of fermentation of glucose, the author goes on to that of fructose, mannose, galactose and then the multisaccharides like maltose, trehalose, sucrose, lactose, melibiose, raffinose, etc. However, in the absence of external substrate yeast cells carry on an endogenous respiration in which the volume of O_2 absorbed equals that of CO_2 evolved (R.Q.=1). The mechanism involved in this case is of a different nature and is explained in chapter IV. The next three chapters deal with assimilation of Carbon, and with Nitrogen and Sulphur metabolism. Then follow growth, reproduction and the genetical aspects including induced mutations. The chapter entitled "The yeast colony in practice" gives a very readable account of the culturing and preservation of yeasts. Finally, there is an ecological essay in which attention is drawn to the occurrence of yeasts in air, water, brine and soil. It is interesting to learn that yeasts sometimes gain access, through wounds, to the interior of plants causing fermentation in the milk of damaged coconuts. In sugarcane they may enter internal cavities through splits in the sheathing leaf-bases and provide a heavy infection in the crude pressed juice. The nectaries of flowers often contain yeasts capable of growing at high sugar concentrations and there are forms in honey which can adjust themselves to a sugar concentration of up to 75 per cent weight per volume. Finally, there are a couple of genera which cause important plant diseases and several affect even human beings.

Altogether, Dr. Ingram's book brings together a lot of information which was previously available only in widely scattered journals. While the treatment is predominantly biochemical (and justifiably so), other aspects like growth, reproduction, genetics, variation and ecology have not been neglected. The book is a valuable reference work for all botanists and plant physiologists.

P. MAHESHWARI

DE ROBERTIS, E. D. P., NOWINSKI, W. W. & SAEZ, F. A. 1954. "General Cytology." 2nd Ed. Pp. 456. W.B. Saunders Company, Philadelphia. $ 7.75.

TWENTY-FIVE years ago cytology was little more than an oil immersion study of the cell. In recent years, with newer techniques, there has been a big change in our outlook. Now we are concerned with submicroscopic organization which deals with the architectural arrangement of the molecules and micelles of living matter. The modern cytologist is not content with a mere description of the changes in the cell but seeks an explanation of these changes in terms of physics and chemistry. Thus the so-called classical cytology is being superseded by a new dynamic cytology with considerable emphasis on the physico-chemical and metabolic processes taking place in the cytoplasm.

The book under review is an excellent summary of such work. The following selected chapter headings give some idea of the scope of the work: Sub-microscopic organization of the cell; Morphology and functional significance of the cytoplasmic organoids; Plasmic membrane and cell permeability; Structure and cytochemistry of the nucleus in the interphasic state; Enzymes and cell metabolism; Cytological and cytochemical manifestations of cellular activity; Differentiation, senescence and death of the cell. Chromosome structure, mitosis, meiosis and sex do receive consideration but the emphasis lies on the topics mentioned above.

The book is clearly written and adequately illustrated and is sure to attract many teachers and students who wish to know more about the complexities of the functional aspects of the cell as learnt from the use of the phase and electron microscope, microdissection, tissue culture, vital and supravital staining, and other

methods. Most of the examples are zoological but the methods being the same the book is of equal interest to the botanist.

P. MAHESHWARI

WATSON, E. V. 1955. "British Mosses and Liverworts." Pp. 419. Cambridge University Press. 45*s*.

IT is estimated that there are about 1500 spermatophytes and 900 bryophytes in the British Isles. Due to their large size and greater economic importance it is natural that the former have received a great deal of attention. The bryophytes, small and inconspicuous though they are, are objects of such beauty when seen under the stereoscopic microscope that a new book on the subject is most welcome. As the title shows, the approach is mainly taxonomic. The arrangement and nomenclature follows that of Richards for mosses and Evans for liverworts. The descriptions are clear and suitably illustrated with line drawings. There are 18 magnificent plates giving photographs of *Polytrichum, Fissidens, Leucobryum, Grimmia, Rhacomitrium, Mnium, Thammium, Thuidium, Hypnum, Plagiochila, Marchantia, Conocephalum, Lunularia* and some others.

The introductory chapter of the book gives a general account of the external morphology of the bryophytes, methods of collection and preservation, and the difference between mosses and liverworts. At the end of the book there are lists giving an idea of the range of species likely to be encountered in different habitats. While the rarer species have been omitted, the book deals with all the common liverworts and mosses of the U.K. and there is in each case a brief note on ecology immediately after the description of the species. This may be illustrated by the following paragraph on *Porella platyphylla*: "It is mainly a plant of chalk and limestone districts, where it will grow on rocks, tree roots and soil. It demands some shade and is sometimes the chief hepatic on the ground in beech woods on chalk. On banks and the bases of stone walls a commonly associated species is the moss *Anomodon viticulosus*."

The book ought to stimulate further work on the group, particularly the ecological aspect which has acquired considerable importance in recent years.

P. MAHESHWARI

WARDLAW, C. W. 1955. "Embryogenesis in Plants." Pp. 381. Methuen & Co. Ltd., London. 42*s*.

"EMBRYOGENESIS IN PLANTS" is an adequately documented, well illustrated, thought-provoking treatise. It brings together observations and conclusions on the embryogeny of all groups of plants, from algae to angiosperms. The genetical, physical and physiological factors, experimental work as applied to the investigation of embryos, and relationships have been considered. It is indeed a timely survey which will promote a better understanding of the "parallelisms of development". As the author says, "Studies of embryogenesis, therefore, not only carry their own interest; they should be regarded as the primary approach to the study of morphogenesis".

Recent discoveries in the field of genetics, biochemistry, physiology and culture technique have somewhat helped us to understand the factors which determine the development of the embryo. It is probably through biochemical effects that the gametophyte controls the maturation of the egg and subsequent growth of the embryo. Worthy of note are the experiments of Whitaker, Knapp, Hurd, Lowrance, Lillie, Conklin, Schechter and others on the effect of light, temperature, *p*H gradient, centrifuging, electric current, etc., on the polarity of the zygotes of *Fucus*, *Cystoseira* (Phaeophyceae), and *Griffithsia* (Rhodophyceae). Polarity seems to be considerably affected by external factors and "among these, gradients of specific substances are important". We still need more facts for a clearer understanding as to how polarity can be controlled.

It is interesting that in the algae, the early determination of polarity and axial development take place in a purely aquatic environment independently of parental nutrition. In green and some of the simpler brown and red algae the

embryogeny is hardly comparable to that of the land plants. However, in some types it affords close parallelisms with the embryogeny of higher plants and their inclusion in the group ' Embryophyta ' appears justified.

In bryophytes if the unfertilized egg is regarded as a homogeneous reaction system, then the stimulus of fertilization and the impact of acropetal gradients might explain the transition to a heterogeneous system. The metabolic complex at the apex of the zygote is different from that at its base and the hypobasal region, which is closest to the source of nutrition, undergoes limited growth whereas the upper end remains embryonic and capable of continued growth. The fertilized ovum may itself be a source of growth-regulating substances. Wardlaw explains that the sequence of distinctive histological developments is determined by a sequence of changes in the underlying reaction system. On genetical basis it may be due to the fact that different genes, or sets of genes, are successively evoked during the ontogenic development of the sporophyte. The presence of chlorophyll in the sporophytes of *Riccia* and *Ricciocarpus* indicates that these are simple rather than primitive. Studhalter grew excised young sporophytes of *Sphaerocarpus* and *Riella* in water and they reached full maturity through their own photosynthetic activity.

In *Lycopodium*, some species of which are protocormous, the difference in the growth of the embryo is explained on the basis that if the environmental conditions are favourable and nutrition is balanced, there is continued meristematic activity at the shoot apex giving rise to an elongating leafy shoot. On the other hand, if the embryo does not receive balanced nutrition, unfavourable environmental conditions modify the character of growth in different regions of the embryo. The embryonic development in *Selaginella* depends on the distribution of specific metabolites. The distal end of the embryo secretes enzymes which digest the adjoining tissue like the digestion of the endosperm by the embryo of flowering plants. In this genus the suspensor is of phylogenetic and morphogenetic interest and in some species it may be absent. For example, in *S. kraussiana*, the archegonium elongates into a tube-like structure (embryoschlauch) which penetrates deeply into the prothallial tissue with the embryo lying at its extremity. In *Isoetes* there is no suspensor and Bower's assumption that the zygote has become ' inverted ' as a result of the loss or absence of this structure cannot be explained on the basis of a heterogeneous distribution of metabolites towards the two poles of the zygote. In the ferns it is probable that a heterogeneous distribution of the metabolites takes place even in the unfertilized egg and a basipetal auxin gradient and an acropetal gradient of carbohydrates and other metabolites may cause physiological differences on opposite sides of the ovum. The difference in development of the gametophyte and sporophyte is not merely a matter of chromosome number, but there must be some overriding metabolic difference, or a difference in the biochemical constitution of the spore and the zygote.

In gymnosperms also there is a basipetal physiological gradient in the female gametophyte which may determine the polarity and orientation of the proembryo and subsequently of the embryo. At the time of fertilization there is a metabolic heterogeneity in the egg cell. Cleavage polyembryony appears to be due to the action of gene-controlled enzymes.

The embryo sac in angiosperms is considered to be a biochemical and biophysical system. There is a gradient of nutrients from the base of the embryo sac to its micropylar end and the starch content becomes maximal about the time of fertilization. The physiological conditions at the micropylar end and in the centre of the embryo sac are considered as important. Syngamy is followed by a period of rest during which there is reorganization of the cytopasm of the egg cell and polarity is perhaps determined either in the ovum or the undivided zygote. The histological pattern is probably indicative of different metabolic activity in the embryo and segmentation is gene-determined. However, at maturity, in the majority of plants — monocotyledons or dicotyledons—the embryo exhibits great similarity in structure. Reference has been made to the work of Marrè and Murneek who have

shown that one effect of fertilization in maize is that hormones are produced and these have a regulating action on the movement of carbohydrates and nitrogen-containing metabolites into the ovule. More or less similar results could be produced by artificial application of hormones.

With further study of embryogenesis we may look for greater success in the artificial cultures of embryos. A knowledge of the forms in which nitrogen can be utilized by the embryo is of paramount importance. Studies with orchid embryos indicate that, as compared to amino acids, ammonium nitrate appears to be better utilized. Whereas some amino acids were stimulatory, others were inhibitory.

In the last chapter, the author has very ably summarized what has been said before in the earlier chapters. The ultimate aim of the study of embryogenesis is to explain how orderly morphological and histological developments are brought about. It is but natural that in an attempt to explain the causes of embryogenesis the author has made many speculations. These should stimulate all workers in the field.

The book is very nicely got up, but it suffers from a few misprints here and there, e.g. Bowar for Bower (p. 13), organis for organs (p. 105), Thathacher for Thathachar (p. 262), ovulo for ovule (p. 308), Kapie for Kapil (p. 349), etc. The choice of figure 66D (p. 263) is not a happy one since vertical division of the zygote in *Euphorbia rothiana*, as claimed by Srivastava (1952), is probably incorrect. These defects, however, do not materially detract from the value of the book.

B. M. Johri

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PLANT BREEDING ABSTRACTS is produced and edited by the Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge, England, on behalf of the Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Nr. Slough, Bucks. Subscription rates are 60s. per volume (with subject index), less 20 per cent to subscribers in the British Commonwealth (other than recognized booksellers) who send their subscriptions direct. Orders may be placed through booksellers or sent to

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LA CELLULE

JOURNAL OF ORIGINAL INVESTIGATIONS IN CYTOLOGY AND HISTOLOGY

Founded by

J. B. CARNOY IN 1884

Edited by

PROFESSORS P. DEBAISIEUX (ZOOLOGY) AND P. MARTENS (BOTANY AND CYTOLOGY), LOUVAIN, BELGIUM

Three numbers constitute a volume — generally annual — of 400 to 450 pages quarto with numerous double-page plates and text-figures. Volume 57 (1955) in course of publication.

Subscription price per volume: frs b. 550 (= $ 11.00) post paid. Address the Editor: C. UYSTPRUYST, LIBRAIRIE UNIVERSITAIRE, RUE DE LA MONNAIE, LOUVAIN, BELGIUM.

General indexes for volumes 1 to 40 (1885-1931) and author index for volumes 41-50 (1932-1946): 110 pages quarto ; price separately: frs b. 100 (= $ 2.00).

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